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## COMPOSITIONS AND METHODS FOR THE TREATMENT OF TUMOR

Related Applications

This is a non-provisional application filed under 37 C.F.R. 1.53(b)(1), claiming priority under 35 U.S.C. 119(e) to provisional application nos.: serial no. 60,088,742, filed 10 June, 1998; serial no. 60/086,414 filed 22 May 1998, serial no. 60,070,440, filed 05 January 1998; serial no. 60/109,304 filed 20 November 1998; serial no. 60/083,500 filed 29 April 1998; and serial no. "to be assigned" (Attorney Docket No. PR1533), filed 10 November 1998 the contents of which are incorporated herein by reference.

Field of the Invention

The present invention relates to compositions and methods for the diagnosis and treatment of tumor.

Background of the Invention

Malignant tumors (cancers) are the second leading cause of death in the United States, after heart disease (Boring et al., CA Cancer J. Clin., 43, 7, [1993]).

Cancer is characterized by the increase in the number of abnormal, or neoplastic, cells derived from a normal tissue which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells which eventually spread via the blood or lymphatic system to regional lymph nodes and to distant sites (metastasis). In a cancerous state a cell proliferates under conditions in which normal cells would not grow. Cancer manifests itself in a wide variety of forms, characterized by different degrees of invasiveness and aggressiveness.

Alteration of gene expression is intimately related to the uncontrolled cell growth and differentiation which are a common feature of all cancers. The genomes of certain well studied tumors have been found to show decreased expression of recessive genes, usually referred to as tumor suppression genes, which would normally function to prevent malignant cell growth, and/or overexpression of certain dominant genes, such as oncogenes, that act to promote malignant growth. Each of these genetic changes appears to be responsible for importing some of the traits that, in aggregate, represent the full neoplastic phenotype (Hunter, Cell 64, 1129 [1991]; Bishop, Cell 64, 235-248 [1991]).

A well known mechanism of gene (e.g. oncogene) overexpression in cancer cells is gene amplification. This is a process where in the chromosome of the ancestral cell multiple copies of a particular gene are produced. The process involves unscheduled replication of the region of chromosome comprising the gene, followed by recombination of the replicated segments back into the chromosome (Alitalo et al., Adv. Cancer Res. 47, 235-281 [1986]). It is believed that the overexpression of the gene parallels gene amplification, i.e. is proportionate to the number of copies made.

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. For example, it has been found that the human ErbB2 gene (*erbB2*, also known as *her2*, or *c-erbB-2*), which encodes a 185-kd transmembrane glycoprotein receptor (p185<sup>HER2</sup>, HER2) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., Science 235:177-182 [1987]; Slamon et al., Science 244:707-712 [1989]).

It has been reported that gene amplification of a proto-oncogene is an event typically involved in the more malignant forms of cancer, and could act as a predictor of clinical outcome (Schwab *et al.*, Genes Chromosomes Cancer 1, 181-193 [1990]; Alitalo *et al.*, *supra*). Thus, *erbB2* overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon *et al.*, [1987] and [1989], *supra*; Ravdin and Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochim Biophys Acta* 1198:165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluorouracil) and anthracyclines (Baselga *et al.*, *Oncology* 11(3 Suppl 1):43-48 [1997]). However, despite the association of *erbB2* overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (*Ibid*). A recombinanthumanized anti-ErbB2 (anti-HER2) monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or Herceptin<sup>®</sup>) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anticancer therapy. (Baselga *et al.*, *J. Clin. Oncol.* 14:737-744 [1996]).

#### 15 Summary of the Invention

The present invention concerns compositions and methods for the diagnosis and treatment of neoplastic cell growth and proliferation in mammals, including humans. The present invention is based on the identification of genes that are amplified in the genome of tumor cells. Such gene amplification is expected to be associated with the overexpression of the gene product and contribute to tumorigenesis. Accordingly, the proteins encoded by the amplified genes are believed to be useful targets for the diagnosis and/or treatment (including prevention) of certain cancers, and may act of predictors of the prognosis of tumor treatment.

In one embodiment, the present invention concerns an isolated antibody which binds a polypeptide which is designated PRO201 (UNQ175), PRO292 (UNQ255), PRO327 (UNQ288), PRO1265 (UNQ636), PRO344 (UNQ303), PRO343 (UNQ302), PRO347 (UNQ306), PRO357 (UNQ314), PRO715 (UNQ383), PRO1017 (UNQ500), PRO1112 (UNQ555), PRO509 (UNQ329), PRO853 (UNQ419) or PRO882 (UNQ448), wherein "PRO" stands for "protein" and "UNQ" stands for "unique". Throughout this specification, the "PRO" and "UNQ" numbers are used interchangeably to designate the respective proteins, wherein UNQ designates the native human sequence only and PRO designates the native sequence and active variants thereof. In one aspect, the antibody induces death of a cell overexpressing a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide. In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.

In another embodiment, the invention concerns a composition comprising an antibody which binds a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. In

another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

In a further embodiment, the invention concerns nucleic acid encoding an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715,  
5 anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody, and vectors and recombinant host cells comprising such nucleic acid.

In a still further embodiment, the invention concerns a method for producing an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715,  
10 anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody by culturing a host cell transformed with nucleic acid encoding the antibody under conditions such that the antibody is expressed, and recovering the antibody from the cell culture.

The invention further concerns antagonists and agonists of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide that inhibit one or more of the functions or activities of the PRO201, PRO292, PRO327,  
15 PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

In a further embodiment, the invention concerns isolated nucleic acid molecules that hybridize to the complement of the nucleic acid molecules encoding the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides.  
20 The nucleic acid preferably is DNA, and hybridization preferably occurs under stringent conditions. Such nucleic acid molecules can act as antisense molecules of the amplified genes identified herein, which, in turn, can find use in the modulation of the respective amplified genes, or as antisense primers in amplification reactions. Furthermore, such sequences can be used as part of ribozyme and/or triple helix sequence which, in turn, may be used in regulation of the amplified genes.

In another embodiment, the invention concerns a method for determining the presence of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide comprising exposing a cell suspected of containing the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide to an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-  
30 PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and determining binding of the antibody to the cell.

In yet another embodiment, the present invention concerns a method of diagnosing tumor in a mammal, comprising detecting the level of expression of a gene encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or  
35 PRO882 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher expression level in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing tumor in a mammal, comprising (a) contacting an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-

PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide in the test sample. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art.

The test sample is usually obtained from an individual suspected to have neoplastic cell growth or proliferation (e.g. cancerous cells).

In another embodiment, the present invention concerns a cancer diagnostic kit, comprising an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and a carrier (e.g. a buffer) in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

In yet another embodiment, the invention concerns a method for inhibiting the growth of tumor cells comprising exposing a cell which overexpresses a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide to an effective amount of an agent inhibiting the expression and/or activity of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide. The agent preferably is an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody, a small organic and inorganic molecule, peptide, phosphopeptide, antisense or ribozyme molecule, or a triple helix molecule. In a specific aspect, the agent, e.g. anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody induces cell death. In a further aspect, the tumor cells are further exposed to radiation treatment and/or a cytotoxic or chemotherapeutic agent.

In a further embodiment, the invention concerns an article of manufacture, comprising:

a container;

a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overexpression of a PRO201, PRO292, PRO327,

PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, and the active agent in the composition is an agent inhibiting the expression and/or activity of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide. In a preferred aspect, the active agent is an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody.

A method for identifying a compound capable of inhibiting the expression and/or activity of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, comprising contacting a candidate compound with a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide under conditions and for a time sufficient to allow these two components to interact. In a specific aspect, either the candidate compound or the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide is immobilized on a solid support.

#### Brief Description of the Figures

Figure 1 (SEQ ID NO: 1) is the nucleotide sequence of DNA30676 encoding a PRO201 (UNQ175).

Figure 2 (SEQ ID NO: 2) is the amino acid sequence of the native human PRO201 (UNQ175) polypeptide.

Figure 3 (SEQ ID NO: 3) is the nucleotide sequence of DNA35617 encoding a PRO292.

Figure 4 (SEQ ID NO: 4) is the amino acid sequence of the native human PRO292 (UNQ266) polypeptide.

Figure 5 (SEQ ID NO: 5) is the nucleotide sequence of DNA38113 encoding a PRO327.

Figure 6 (SEQ ID NO: 6) is the amino acid sequence of the native human PRO327 (UNQ288) polypeptide.

Figure 7 (SEQ ID NO: 7) is the nucleotide sequence of DNA60764 encoding a PRO1265.

Figure 8 (SEQ ID NO: 8) is the amino acid sequence of the native human PRO1265 (UNQ636) polypeptide.

Figure 9 (SEQ ID NO: 9) is the nucleotide sequence of DNA40592 encoding a PRO344.

Figure 10 (SEQ ID NO: 10) is the amino acid sequence of the native human PRO344 (UNQ303) polypeptide.

Figure 11 (SEQ ID NO: 11) is the nucleotide sequence of DNA43318 encoding a PRO343.

Figure 12 (SEQ ID NO: 12) is the amino acid sequence of the native human PRO343 (UNQ302) polypeptide.

Figure 13 (SEQ ID NO: 13) is the nucleotide sequence of DNA44176 encoding a PRO347.

Figure 14 (SEQ ID NO: 14) is the amino acid sequence of the native human PRO347 (UNQ306) polypeptide.

Figure 15 (SEQ ID NO: 15) is the nucleotide sequence of DNA44804 encoding a PRO357.

Figure 16 (SEQ ID NO: 16) is the amino acid sequence of the native human PRO357 (UNQ314) polypeptide.

Figure 17 (SEQ ID NO: 17) is the nucleotide sequence of DNA52722 encoding a PRO715.

Figure 18 (SEQ ID NO: 18) is the amino acid sequence of the native human PRO715 (UNQ383) polypeptide.

Figure 19 (SEQ ID NO: 19) is the nucleotide sequence of DNA56112 encoding a PRO1017.

5 Figure 20 (SEQ ID NO: 20) is the amino acid sequence of the native human PRO1017 (UNQ500) polypeptide.

Figure 21 (SEQ ID NO: 21) is the nucleotide sequence of DNA57702 encoding a PRO1112.

Figure 22 (SEQ ID NO: 22) is the amino acid sequence of the native human PRO1112 (UNQ555).

Figure 23 (SEQ ID NO: 23) is the nucleotide sequence of DNA50148 encoding a PRO509.

10 Figure 24 (SEQ ID NO: 24) is the amino acid sequence of the native human PRO509 polypeptide (UNQ329).

Figure 25 (SEQ ID NO: 25) is the nucleotide sequence of DNA48227 encoding a PRO1350.

Figure 26 (SEQ ID NO: 26) is the amino acid sequence of the native human PRO1350 polypeptide (UNQ419).

15 Figure 27 (SEQ ID NO: 27) is the nucleotide sequence of DNA58125 encoding a PRO882.

Figure 28 (SEQ ID NO: 28) is the amino acid sequence of the native human PRO882 polypeptide (UNQ448).

Figure 29 (SEQ ID NO: 29) is the nucleotide sequence of DNA28710 used in the cloning of DNA 30676 encoding PRO201.

20 Figure 30 (SEQ ID NO: 30) is the nucleotide sequence encoding EST 2452972.

Figure 31 (SEQ ID NO: 31) is the nucleotide sequence encoding EST2099855.

Figure 32 is a map of chromosome 19 showing the mapping regions of DNA30676, DNA38113 and DNA60764.

Figure 33 is a map of chromosome 11 showing the mapping region of DNA354617.

25 Figure 34 is a map of chromosome 16 showing the mapping region of DNA58125 and DNA43318.

Figure 35 is a map of chromosome 7 showing the mapping region of DNA56112.

Figure 36A is map of chromosome 17 showing the mapping region of DNA52722.

Figure 36B is a map of chromosome 17 showing the mapping region of DNA48227.

Figures 37-39 are in situ hybridizations as described in the example section

30 Figure 40 is a map of chromosome 16 showing the mapping region of DNA44804

### Detailed Description of the Invention

#### I. Definitions

The phrases "gene amplification" and "gene duplication" are used interchangeably and refer to a process by which multiple copies of a gene or gene fragment are formed in a particular cell or cell line. The duplicated region (a stretch of amplified DNA) is often referred to as "amplicon." Usually, the amount of the messenger RNA (mRNA) produced, i.e. the level of gene expression, also increases in the proportion of the number of copies made of the particular gene expressed.



"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, prostate cancer, colon cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

"Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In tumor (e.g. cancer) treatment, a therapeutic agent may directly decrease the pathology of tumor cells, or render the tumor cells more susceptible to treatment by other therapeutic agents, e.g. radiation and/or chemotherapy.

The "pathology" of cancer includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, metastasis, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of inflammatory or immunological response, *etc.*

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONIC<sup>TM</sup>.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup> and Re<sup>186</sup>), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytosine, taxoids, e.g. paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, France), taxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), 5-FU, 6-thioguanine, 6-mercaptopurine, actinomycin D, VP-16, chlorambucil, melphalan, and other related nitrogen mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

"Doxorubicin" is an anthracycline antibiotic. The full chemical name of doxorubicin is (8S-cis)-10-[(3-amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexapyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- $\alpha$  and - $\beta$ ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- $\beta$ ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- $\alpha$  and TGF- $\beta$ ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- $\alpha$ , - $\beta$ , and - $\gamma$ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 $\alpha$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF- $\alpha$  or TNF- $\beta$ ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g. Wilman, "Prodrugs in Cancer Chemotherapy", Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting, Belfast (1986), and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery", Directed Drug delivery, Borchardt *et al.*, (ed.), pp. 147-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs,  $\beta$ -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrugs form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

As used herein, the terms "PRO201," "PRO292," "PRO327," "PRO1265," "PRO344," "PRO343," "PRO347," "PRO357," "PRO715," "PRO1017," "PRO1112," "PRO509," "PRO853" or "PRO882" or "PRO201 polypeptide", "PRO292 polypeptide", "PRO327 polypeptide", "PRO1265 polypeptide", "PRO344 polypeptide", "PRO343 polypeptide", "PRO347 polypeptide", "PRO357 polypeptide", "PRO715 polypeptide", "PRO1017 polypeptide", "PRO1112 polypeptide", "PRO509 polypeptide", "PRO853 polypeptide" or "PRO882 polypeptide) encompass both native sequence and active variants thereof. The PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic means. The term specifically encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide. Fragments of the respective native polypeptides herein include, but are not limited, to polypeptide variants from which the native N-terminal signal sequence has been fully or partially deleted or replaced by another sequence, and extracellular domains of the respective native sequences, regardless whether such truncated (secreted) forms occur in nature. Fragments are preferably sufficient in length for the production of an antibody specifically binding the corresponding native "PRO" polypeptide.

As used herein, the terms "UNQ175", "UNQ255", "UNQ288", "UNQ636", "UNQ303", "UNQ302", "UNQ306", "UNQ314", "UNQ383", "UNQ500", "UNQ555", "UNQ329", "UNQ419" and "UNQ448" are used to refer to the native sequence polypeptide corresponding to the PRO designated sequences above appearing as a full-length native presequence or a mature form of: a PRO201 polypeptide shown in Figure 2 (SEQ ID NO: 2); a PRO292 polypeptide shown in Figure 4 (SEQ ID NO: 4); a PRO327 polypeptide shown in Figure 6 (SEQ ID NO: 6); a PRO1265 polypeptide shown in Figure 8 (SEQ ID NO: 8); a PRO344 polypeptide shown in Figure 10 (SEQ ID NO: 10); a PRO343 polypeptide shown in Figure 12 (SEQ ID NO: 12); a PRO347 polypeptide shown in Figure 14 (SEQ ID NO: 14); a PRO357 polypeptide shown in Figure

16 (SEQ ID NO: 16); a PRO715 polypeptide shown in Figure 18 (SEQ ID NO: 18); a PRO1017 polypeptide shown in Figure 20 (SEQ ID NO: 20); a PRO1112 polypeptide shown in Figure 22 (SEQ ID NO: 22); a PRO509 polypeptide shown in Figure 24 (SEQ ID NO: 24); a PRO853 polypeptide shown in Figure 26 (SEQ ID NO: 26); and a PRO882 polypeptide shown in Figure 28 (SEQ ID NO: 28), respectively. Said another way, the "UNQ" designation refers to the specific native sequence, while the "PRO" designation refers to the native sequence and active variants thereof.

An "isolated" nucleic acid molecule encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid. An isolated PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide includes nucleic acid molecules contained in cells that ordinarily express PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotic cells, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal

when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and % SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

"Active" or "activity" in the context of molecules identified based upon the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides (or their coding sequences) refers to polypeptides (e.g. antibodies) or organic or inorganic small molecules, peptides, etc. which retain the biological and/or immunological activities/properties of a native or naturally-occurring PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

"Biological activity" in the context of an antibody or another molecule that can be identified by the screening assays disclosed herein (e.g. an organic or inorganic small molecule, peptide, *etc.*) is used to refer to the ability of such molecules to bind or complex with the polypeptides encoded by the amplified genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. A preferred biological activity is growth inhibition of a target tumor cell. Another preferred biological activity is cytotoxic activity resulting in the death of the target tumor cell.

The phrase "immunological property" means immunological cross-reactivity with at least one epitope of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

"Immunological cross-reactivity" as used herein means that the candidate polypeptide is capable of competitively inhibiting the qualitative biological activity of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide having this activity with polyclonal antisera raised against the known active PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide. Such antisera are prepared in conventional fashion by injecting goats or rabbits, for example, subcutaneously with the known active analogue in complete Freund's adjuvant, followed by booster intraperitoneal or subcutaneous injection in incomplete Freund's. The immunological cross-reactivity preferably is "specific", which means that the binding affinity of the immunologically cross-reactive molecule (e.g. antibody) identified, to the corresponding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide is significantly higher (preferably at least about 2-times, more preferably at least about 4-times, even more preferably at least about 8-times, most preferably at least about 8-times higher) than the binding affinity of that molecule to any other known native polypeptide.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO201; PRO292; PRO327; PRO1265; PRO344; PRO343; PRO347; PRO357; PRO715; PRO1017; PRO1112; or PRO509 polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, small organic molecules, etc.

A "small molecule" is defined herein to have a molecular weight below about 500 Dalton.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal

antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 Dalton, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ( $V_H$ ) followed by a number of constant domains. Each light chain has a variable domain at one end ( $V_L$ ) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a  $\beta$ -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, *NIH Publ. No. 91-3242*, Vol. I, pages 647-669 (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" to "CDR" (i.e. residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD. [1991]) and/or those residues from a "hypervariable loop" (i.e. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Clothia and Lesk, *J. Mol. Biol.* **196**:901-917 [1987]). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Eng.* **8**(10):1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an  $F(ab')_2$  fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

- 5 "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the  $V_H$ - $V_L$  dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three  
10 CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

- The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge  
15 region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group.  $F(ab')_2$  antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

- The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their  
20 constant domains.

- Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes  
25 of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

- The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal  
30 antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal"  
35 indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 [1975], or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from



phage antibody libraries using the techniques described in Clackson *et al.*, *Nature*, 352: 624-628 [1991] and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 [1984]).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332: 323-329 [1988]; and Presta, *Curr. Opin. Struct. Biol.*, 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

"Single-chain Fv" or "sFv" antibody fragments comprise the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) in the same polypeptide chain (V<sub>H</sub> - V<sub>L</sub>). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials

which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. Radionuclides that can serve as detectable labels include, for example, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, and Pd-109.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as an PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, or an antibody thereto and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

## II. Compositions and Methods of the Invention

- I. Preparation of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO201 (UNQ175), PRO292 (UNQ255), PRO327 (UNQ288), PRO1265 (UNQ636), PRO344 (UNQ303), PRO343 (UNQ302), PRO347 (UNQ306), PRO357 (UNQ314), PRO715 (UNQ383), PRO1017 (UNQ500), PRO1112 (UNQ555), PRO509 (UNQ329), PRO853 (UNQ419) or PRO882 (UNQ448). In particular, cDNAs encoding certain PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. In the present specification the proteins encoded by nucleic acid referred to as "DNA30676", "DNA35617", "DNA38113", "DNA60764", "DNA40592", "DNA43318", "DNA44176", "DNA44804", "DNA52722", "DNA56112", "DNA57702", "DNA50148", "DNA48227", "DNA58125" are referred to as UNQ175, UNQ255, UNQ288, UNQ636, UNQ303, UNQ302, UNQ306, UNQ314, UNQ383, UNQ500, UNQ555, UNQ329, UNQ419 or UNQ448, respectively. However, the above sequences including further native homologues and variants are included in the definition of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, regardless of their origin or mode of expression.

The description below relates primarily to production of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides by culturing cells transformed or transfected with a vector containing PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides. For instance, the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart *et al.*, *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.*, **85**: 2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

a. Isolation of DNA Encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

DNA encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 may be obtained from a cDNA library prepared

from tissue believed to possess the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 mRNA and to express it at a detectable level. Accordingly, human PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 DNA can be conveniently obtained  
 5 from a cDNA library prepared from human tissue, such as described in the Examples. PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene  
 10 encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives  
 20 are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known  
 25 sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined through sequence alignment using computer software programs such as ALIGN, DNASTAR, and INHERIT which employ various algorithms to measure homology.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or  
 30 genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

#### b. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for  
 35 PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the

productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example, CaPO<sub>4</sub> and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, **23**:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, **52**:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, **130**:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, **76**:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, **185**:527-537 (1990) and Mansour *et al.*, *Nature*, **336**:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635).

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism.

Suitable host cells for the expression of glycosylated PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen. Virol.*, **36**:59 (1977)); Chinese hamster ovary cells-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, **77**:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, **23**:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

#### c. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 may

be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*,

10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO201-, PRO292-, PRO327-, PRO1265-,

PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

d. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 DNA and encoding a specific antibody epitope.

e. Purification of Polypeptide

Forms of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.



It may be desired to purify PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide produced.

2. Amplification of Genes Encoding the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 Polypeptides in Tumor Tissues and Cell Lines

The present invention is based on the identification and characterization of genes which are amplified in certain cancer cells.

The genome of prokaryotic and eukaryotic organisms is subjected to two seemingly conflicting requirements. One is the preservation and propagation of DNA as the genetic information in its original form, to guarantee stable inheritance through multiple generations. On the other hand, cells or organisms must be able to adapt to lasting environmental changes. The adaptive mechanisms can include qualitative or quantitative modifications of the genetic material. Qualitative modifications include DNA mutations, in which coding sequences are altered resulting in a structurally and/or functionally different protein. Gene amplification is a quantitative modification, whereby the actual number of complete coding sequence, *i.e.* a gene, increases, leading to an increased number of available templates for transcription, an increased number of translatable transcripts, and, ultimately, to an increased abundance of the protein encoded by the amplified gene.

The phenomenon of gene amplification and its underlying mechanisms have been investigated *in vitro* in several prokaryotic and eukaryotic culture systems. The best-characterized example of gene amplification involves the culture of eukaryotic cells in medium containing variable concentrations of the cytotoxic drug methotrexate (MTX). MTX is a folic acid analogue and interferes with DNA synthesis by blocking the enzyme dihydrofolate reductase (DHFR). During the initial exposure to low concentrations of MTX most cells (>99.9%) will die. A small number of cells survive, and are capable of growing in increasing concentrations of MTX by producing large amounts of DHFR-RNA and protein. The basis of this overproduction is the amplification of the single DHFR gene. The additional copies of the gene are found as extrachromosomal copies in the form of small, supernumerary chromosomes (double minutes) or as integrated chromosomal copies.

Gene amplification is most commonly encountered in the development of resistance to cytotoxic drugs (antibiotics for bacteria and chemotherapeutic agents for eukaryotic cells) and neoplastic transformation. Transformation of a eukaryotic cell as a spontaneous event or due to a viral or chemical/environmental insult is typically associated with changes in the genetic material of that cell. One of the most common genetic changes observed in human malignancies are mutations of the p53 protein. p53 controls the transition of cells from the stationary (G1) to the replicative (S) phase and prevents this transition in the presence of DNA damage. In other words, one of the main consequences of disabling p53 mutations is the accumulation and propagation of DNA damage, *i.e.* genetic changes. Common types of genetic changes in neoplastic cells are, in addition to point mutations, amplifications and gross structural alterations, such as translocations.

The amplification of DNA sequences may indicate specific functional requirement as illustrated in the DHFR experimental system. Therefore, the amplification of certain oncogenes in malignancies points toward a causative role of these genes in the process of malignant transformation and maintenance of the transformed phenotype. This hypothesis has gained support in recent studies. For example, the *bcl-2* protein was found to be amplified in certain types of non-Hodgkin's lymphoma. This protein inhibits apoptosis and leads to the progressive accumulation of neoplastic cells. Members of the gene family of growth factor receptors have been found to be amplified in various types of cancers suggesting that overexpression of these receptors may make neoplastic cells less susceptible to limiting amounts of available growth factor. Examples include the amplification of the androgen receptor in recurrent prostate cancer during androgen deprivation therapy and the amplification of the growth factor receptor homologue ERG2 in breast cancer. Lastly, genes involved in intracellular signaling and control of cell cycle progression can undergo amplification during malignant transformation. This is illustrated by the amplification of the *bcl-1* and *ras* genes in various epithelial and lymphoid neoplasms.

These earlier studies illustrate the feasibility of identifying amplified DNA sequences in neoplasms, because this approach can identify genes important for malignant transformation. The case of ERB2 also demonstrates the feasibility from a therapeutic standpoint, since transforming proteins may represent novel and specific targets for tumor therapy.

Several different techniques can be used to demonstrate amplified genomic sequences. Classical cytogenetic analysis of chromosome spreads prepared from cancer cells is adequate to identify gross structural alterations, such as translocations, deletions and inversions. Amplified genomic regions can only be visualized, if they involve large regions with high copy numbers or are present as extrachromosomal material. While cytogenetics was the first technique to demonstrate the consistent association of specific chromosomal changes with particular neoplasms, it is inadequate for the identification and isolation of manageable DNA sequences. The more recently developed technique of comparative genomic hybridization (CGH) has illustrated the widespread phenomenon of genomic amplification in neoplasms. Tumor and normal DNA are hybridized simultaneously onto metaphases of normal cells and the entire genome can be screened by image analysis for DNA sequences that are present in the tumor at an increased frequency. (WO 93/18,186; Gray *et al.*, *Radiation Res.* 137, 275-289 [1994]). As a screening method, this type of analysis has revealed a large number of recurring amplicons (a stretch of amplified DNA) in a variety of human neoplasms. Although CGH is more sensitive than classical cytogenetic analysis in identifying amplified stretches of DNA, it does not

allow a rapid identification and isolation of coding sequences within the amplicon by standard molecular genetic techniques.

The most sensitive methods to detect gene amplification are polymerase chain reaction (PCR)-based assays. These assays utilize very small amount of tumor DNA as starting material, are exquisitely sensitive, provide DNA that is amenable to further analysis, such as sequencing and are suitable for high-volume throughput analysis.

The above-mentioned assays are not mutually exclusive, but are frequently used in combination to identify amplifications in neoplasms. While cytogenetic analysis and CGH represent screening methods to survey the entire genome for amplified regions, PCR-based assays are most suitable for the final identification of coding sequences, i.e. genes in amplified regions.

According to the present invention, such genes have been identified by quantitative PCR (S. Gelmini *et al.*, *Clin. Chem.* 43:752 [1997]), by comparing DNA from a variety of primary tumors, including breast, lung, colon, prostate, brain, liver, kidney, pancreas, spleen, thymus, testis, ovary, uterus, etc. tumor, or tumor cell lines, with pooled DNA from healthy donors. Quantitative PCR was performed using a TaqMan instrument (ABI). Gene-specific primers and fluorogenic probes were designed based upon the coding sequences of the DNAs.

Human lung carcinoma cell lines include A549 (SRC768), Calu-1 (SRC769), Calu-6 (SRC770), H157 (SRC771), H441 (SRC772), H460 (SRC773), H522 (SRC832), H810 (SRC833), SKMES-1 (SRC774) and SW900 (SRC775), all available from ATCC. Primary human lung tumor cells usually derive from adenocarcinomas, squamous cell carcinomas, large cell carcinomas, non-small cell carcinomas, small cell carcinomas, and broncho alveolar carcinomas, and include, for example, SRC724 (squamous cell carcinoma abbreviated as "SqCCa")(LT1), SRC725 (non-small cell carcinoma, abbreviated as "NSCCa")(LT1a), SRC726 (adenocarcinoma, abbreviated as "AdenoCa")(LT2), SRC727 (adenocarcinoma)(LT3), SRC728 (squamous cell carcinoma)(LT4), SRC729 (adenocarcinoma)(LT6), SRC730 (adenocarcinoma)(LT7), SRC731 (adenocarcinoma)(LT9), SRC732 (squamous cell carcinoma)(LT10), SRC733 (adenocarcinoma)(LT11), SRC734 (adenocarcinoma)(LT12), SRC735 (broncho alveolar carcinoma, abbreviated as "BAC")(LT13), SRC736 (squamous cell carcinoma)(LT15), SRC737 (squamous cell carcinoma)(LT16), SRC738 (squamous cell carcinoma)(LT17), SRC739 (squamous cell carcinoma)(LT18), SRC740 (squamous cell carcinoma)(LT19), SRC741 (lung cell carcinoma, abbreviated as "LCCa")(LT21), SRC811 (adenocarcinoma)(LT22).

Colon cancer cell lines include, for example, ATCC cell lines SW480 (adenocarcinoma, SRCC776), SW620 (lymph node metastasis of colon adenocarcinoma, SRC777), Colo320 (carcinoma, SRCC778), Colo205 (carcinoma, SRC828), HCC2998 (carcinoma, SRC830), HT29 (adenocarcinoma, SRC779), HM7 (carcinoma, SRC780), KM12 (carcinoma, SRC831), CaWiDr (adenocarcinoma, SRC781), HCT15 (carcinoma, SRC829), HCT116 (carcinoma, SRC782), SKCO1 (adenocarcinoma, SRC783), SW403 (adenocarcinoma, SRC784), LS174T (carcinoma, SRC785), and HM7 (a high mucin producing variant of ATCC colon adenocarcinoma cell line LS 174T, obtained from Dr. Robert Warren, UCSF). Primary colon tumors include colon adenocarcinomas designated CT1 (SRC751), CT2 (SRC742), CT3 (SRC743), CT4 (SRC752), CT5 (SRC753), CT6 (SRC754), CT7 (SRC755), CT8 (SRC744), CT9 (SRC756), CT10 (SRC745), CT11

(SRC757), CT12 (SRC746), CT14 (SRC747), CT15 (SRC748), CT16 (SRC749), CT17 (SRC750), CT18 (SRCC758), and DcR3, BACrev, BACfwd, T160, and T159.

Human breast carcinoma cell lines include, for example, HBL100 (SRCC759), MB435s (SRCC760), T47D (SRCC761), MB468 (SRCC762), MB175 (SRCC763), MB361 (SRCC764), BT20 (SRCC765), MCF7 (SRCC766), SKBR3 (SRCC767).

### 3. Tissue Distribution

The results of the gene amplification assays herein can be verified by further studies, such as, by determining mRNA expression in various human tissues.

As noted before, gene amplification and/or gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 NA and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided hereinbelow.

### 4. Chromosome Mapping

If the amplification of a given gene is functionally relevant, then that gene should be amplified more than neighboring genomic regions which are not important for tumor survival. To test this, the gene can be mapped to a particular chromosome, e.g. by radiation-hybrid analysis. The amplification level is then determined at the location identified, and at neighboring genomic region. Selective or preferential amplification at the genomic region to which the gene has been mapped is consistent with the possibility that the gene amplification observed promotes tumor growth or survival. Chromosome mapping includes both framework and epicenter mapping. For further details see e.g., Stewart *et al.*, *Genome Research* 7, 422-433 (1997).

### 5. Antibody Binding Studies

The results of the gene amplification study can be further verified by antibody binding studies, in which the ability of anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 to inhibit the expression of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347,

PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptides on tumor (cancer) cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein (encoded by a gene amplified in a tumor cell) in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tumor sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

#### 6. Cell-Based Tumor Assays

Cell-based assays and animal models for tumors (e.g. cancers) can be used to verify the findings of the gene amplification assay, and further understand the relationship between the genes identified herein and the development and pathogenesis of neoplastic cell growth. The role of gene products identified herein in the development and pathology of tumor or cancer can be tested by using primary tumor cells or cells lines that have been identified to amplify the genes herein. Such cells include, for example, the breast, colon and lung cancer cells and cell lines listed above.

In a different approach, cells of a cell type known to be involved in a particular tumor are transfected with the cDNAs herein, and the ability of these cDNAs to induce excessive growth is analyzed. Suitable cells include, for example, stable tumor cells lines such as, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene) and *ras*-transfected NIH-3T3 cells, which can be transfected with the desired gene, and monitored for tumorigenic growth. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit tumorigenic cell growth by exerting cytostatic or cytotoxic activity on the growth of the transformed cells, or by mediating antibody-dependent cellular cytotoxicity (ADCC). Cells transfected with the coding sequences of the genes identified herein can further be used to identify drug candidates for the treatment of cancer.

In addition, primary cultures derived from tumors in transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous

cell lines from transgenic animals are well known in the art (see, e.g. Small *et al.*, *Mol. Cell. Biol.* 5, 642-648 [1985]).

#### 7. Animal Models

A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of tumors, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them particularly predictive of responses in human patients. Animal models of tumors and cancers (e.g. breast cancer, colon cancer, prostate cancer, lung cancer, *etc.*) include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, e.g., murine models. Such models can be generated by introducing tumor cells into syngeneic mice using standard techniques, e.g. subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, or orthotopic implantation, e.g. colon cancer cells implanted in colonic tissue. (See, e.g. PCT publication No. WO 97/33551, published September 18, 1997).

Probably the most often used animal species in oncological studies are immunodeficient mice and, in particular, nude mice. The observation that the nude mouse with hypo/aplasia could successfully act as a host for human tumor xenografts has lead to its widespread use for this purpose. The autosomal recessive *nu* gene has been introduced into a very large number of distinct congenic strains of nude mouse, including, for example, ASW, A/He, AKR, BALB/c, B10.LP, C17, C3H, C57BL, C57, CBA, DBA, DDD, I/St, NC, NFR, NFS, NFS/N, NZB, NZC, NZW, P, RIII and SJL. In addition, a wide variety of other animals with inherited immunological defects other than the nude mouse have been bred and used as recipients of tumor xenografts. For further details see, e.g. *The Nude Mouse in Oncology Research*, E. Boven and B. Winograd, eds., CRC Press, Inc., 1991.

The cells introduced into such animals can be derived from known tumor/cancer cell lines, such as, any of the above-listed tumor cell lines, and, for example, the B104-1-1 cell line (stable NIH-3T3 cell line transfected with the *neu* protooncogene); ~~non-transformed NIH-3T3 cells~~ Caco-2 (ATCC HTB-37); a moderately well-differentiated grade II human colon adenocarcinoma cell line, HT-29 (ATCC HTB-38), or ~~from tumors and cancers~~. Samples of tumor or cancer cells can be obtained from patients undergoing surgery, using standard conditions, involving freezing and storing in liquid nitrogen (Karmali *et al.*, *Br. J. Cancer* 48: 689-696 [1983]).

Tumor cells can be introduced into animals, such as nude mice, by a variety of procedures. The subcutaneous (s.c.) space in mice is very suitable for tumor implantation. Tumors can be transplanted s.c. as solid blocks, as needle biopsies by use of a trochar, or as cell suspensions. For solid block or trochar implantation, tumor tissue fragments of suitable size are introduced into the s.c. space. Cell suspensions are freshly prepared from primary tumors or stable tumor cell lines, and injected subcutaneously. Tumor cells can also be injected as subdermal implants. In this location, the inoculum is deposited between the lower part of the dermal connective tissue and the s.c. tissue. Boven and Winograd (1991), *supra*.

Animal models of breast cancer can be generated, for example, by implanting rat neuroblastoma cells (from which the *neu* oncogen was initially isolated), or *neu*-transformed NIH-3T3 cells into nude mice, essentially as described by Drebin *et al.* *PNAS USA* 83: 9129-9133 (1986).

Similarly, animal models of colon cancer can be generated by passaging colon cancer cells in animals, e.g. nude mice, leading to the appearance of tumors in these animals. An orthotopic transplant model of human colon cancer in nude mice has been described, for example, by Wang *et al.*, *Cancer Research* 54: 4726-4728 (1994) and Too *et al.*, *Cancer Research* 55, 681-684 (1995). This model is based on the so-called  
5 "METAMOUSE" sold by AntiCancer, Inc. (San Diego, California).

Tumors that arise in animals can be removed and cultured *in vitro*. Cells from the *in vitro* cultures can then be passaged to animals. Such tumors can serve as targets for further testing or drug screening. Alternatively, the tumors resulting from the passage can be isolated and RNA from pre-passage cells and cells isolated after one or more rounds of passage analyzed for differential expression of genes of interest. Such  
10 passaging techniques can be performed with any known tumor or cancer cell lines.

For example, Meth A, CMS4, CMS5, CMS21, and WEHI-164 are chemically induced fibrosarcomas of BALB/c female mice (DeLeo *et al.*, *J. Exp. Med.* 146: 720 [1977]), which provide a highly controllable model system for studying the anti-tumor activities of various agents (Palladino *et al.*, *J. Immunol.* 138: 4023-4032 [1987]). Briefly, tumor cells are propagated *in vitro* in cell culture. Prior to injection into the animals,  
15 the cell lines are washed and suspended in buffer, at a cell density of about  $10 \times 10^6$  to  $10 \times 10^7$  cells/ml. The animals are then infected subcutaneously with 10 to 100  $\mu$ l of the cell suspension, allowing one to three weeks for a tumor to appear.

In addition, the Lewis lung (3LL) carcinoma of mice, which is one of the most thoroughly studied experimental tumors, can be used as an investigational tumor model. Efficacy in this tumor model has been  
20 correlated with beneficial effects in the treatment of human patients diagnosed with small cell carcinoma of the lung (SCCL). This tumor can be introduced in normal mice upon injection of tumor fragments from an affected mouse or of cells maintained in culture (Zupi *et al.*, *Br. J. Cancer* 41: suppl. 4, 309 [1980]), and evidence indicates that tumors can be started from injection of even a single cell and that a very high proportion of infected tumor cells survive. For further information about this tumor model see Zacharski,  
25 *Haemostasis* 16: 300-320 [1986]).

One way of evaluating the efficacy of a test compound in an animal model is implanted tumor is to measure the size of the tumor before and after treatment. Traditionally, the size of implanted tumors has been measured with a slide caliper in two or three dimensions. The measure limited to two dimensions does not accurately reflect the size of the tumor, therefore, it is usually converted into the corresponding volume by  
30 using a mathematical formula. However, the measurement of tumor size is very inaccurate. The therapeutic effects of a drug candidate can be better described as treatment-induced growth delay and specific growth delay. Another important variable in the description of tumor growth is the tumor volume doubling time. Computer programs for the calculation and description of tumor growth are also available, such as the program reported by Rygaard and Spang-Thomsen, *Proc. 6th Int. Workshop on Immune-Deficient Animals*, Wu and  
35 Sheng eds., Basel, 1989, 301. It is noted, however, that necrosis and inflammatory responses following treatment may actually result in an increase in tumor size, at least initially. Therefore, these changes need to be carefully monitored, by a combination of a morphometric method and flow cytometric analysis.

Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing

transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, e.g. baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ  
 5 lines (e.g., Van der Putten *et al.*, *Proc. Natl. Acad. Sci. USA* **82**: 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson *et al.*, *Cell* **56**: 313-321 [1989]); electroporation of embryos (Lo, *Mol. Cell. Biol.* **3**: 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano *et al.*, *Cell* **57**: 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene  
 10 only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, e.g., head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al.*, *Proc. Natl. Acad. Sci. USA* **89**: 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For  
 15 example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry. The animals are further examined for signs of tumor or cancer development.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene  
 20 encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017,  
 25 PRO1112, PRO509, PRO853 or PRO882 polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of  
 30 unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, **51**: 503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li *et al.*, *Cell*, **69**: 915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation  
 35 chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout



animals can be characterized for instance, by their ability to defend against certain pathological conditions and by their development of pathological conditions due to absence of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

- 5           The efficacy of antibodies specifically binding the polypeptides identified herein and other drug candidates, can be tested also in the treatment of spontaneous animal tumors. A suitable target for such studies is the feline oral squamous cell carcinoma (SCC). Feline oral SCC is a highly invasive, malignant tumor that is the most common oral malignancy of cats, accounting for over 60% of the oral tumors reported in this species. It rarely metastasizes to distant sites, although this low incidence of metastasis may merely be a reflection of the short survival times for cats with this tumor. These tumors are usually not amenable to surgery, primarily because of the anatomy of the feline oral cavity. At present, there is no effective treatment for this tumor. Prior to entry into the study, each cat undergoes complete clinical examination, biopsy, and is scanned by computed tomography (CT). Cats diagnosed with sublingual oral squamous cell tumors are excluded from the study. The tongue can become paralyzed as a result of such tumor, and even if the treatment kills the tumor, the animals may not be able to feed themselves. Each cat is treated repeatedly, over a longer period of time. Photographs of the tumors will be taken daily during the treatment period, and at each subsequent recheck. After treatment, each cat undergoes another CT scan. CT scans and thoracic radiographs are evaluated every 8 weeks thereafter. The data are evaluated for differences in survival, response and toxicity as compared to control groups. Positive response may require evidence of tumor regression, preferably with improvement of quality of life and/or increased life span.

In addition, other spontaneous animal tumors, such as fibrosarcoma, adenocarcinoma, lymphoma, chondroma, leiomyosarcoma of dogs, cats, and baboons can also be tested. Of these mammary adenocarcinoma in dogs and cats is a preferred model as its appearance and behavior are very similar to those in humans. However, the use of this model is limited by the rare occurrence of this type of tumor in animals.

25           8.       Screening Assays for Drug Candidates

- Screening assays for drug candidates are designed to identify compounds that bind or complex with the polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, e.g. on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, e.g. a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, e.g. the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, e.g. by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature* 340: 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 9578-9582 (1991)] as disclosed by Chevray and Nathans [*Proc. Natl. Acad. Sci. USA* 89: 5789-5793 (1991)]. Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding gene identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the amplified gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the absence and in the presence of

the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

#### 9. Compositions and Methods for the Treatment of Tumors

The compositions useful in the treatment of tumors associated with the amplification of the genes identified herein include, without limitation, antibodies, small organic and inorganic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, etc. that inhibit the expression and/or activity of the target gene product.

For example, antisense RNA and RNA molecule act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation initiation site, e.g. between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g. Rossi, *Current Biology* 4: 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g. PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed hereinabove and/or by any other screening techniques well known for those skilled in the art.

#### 9.1 Antibodies

Some of the most promising drug candidates according to the present invention are antibodies and antibody fragments which may inhibit the production or the gene product of the amplified genes identified herein and/or reduce the activity of the gene products.

##### i. Polyclonal Antibodies

Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean

trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

ii. Monoclonal Antibodies

5 The anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256: 495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

10 The immunizing agent will typically include the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, including fragments, or a fusion protein of such protein or a fragment thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

25 Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection (ATCC), Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

35 The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody

can. for example, be determined by the Scatchard analysis of Munson and Pollard. *Anal. Biochem.*, 107: 220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### iii. Human and Humanized Antibodies

The anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibodies may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR)

of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature* 321: 522-525 (1986); Riechmann *et al.*, *Nature* 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2: 593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones *et al.*, *Nature*, 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332: 323-327 (1988); Verhoeven *et al.*, *Science*, 239: 1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222: 581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1): 86-95 (1991)). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *BioTechnology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

#### iv. Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO 81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U. S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, glycosidase, glucose oxidase, human lysosyme, human glucuronidase, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases (e.g., carboxypeptidase G2 and carboxypeptidase A) and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase useful for converting drugs derivatized with  $\beta$ -lactams into free drugs; and penicillin amidases, such as penicillin Vamidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes" can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO543, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibodies by techniques well known in the art such as the use of the heterobifunctional cross-linking agents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of the antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g. Neuberger *et al.*, *Nature* 312: 604-608 (1984)).

v. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 [1983]). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10: 3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121: 210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of



the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* **175**: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* **148**(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* **90**: 6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* **152**: 5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* **147**: 60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given "Pro" protein herein. Alternatively, an anti-"PRO" protein arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular "PRO" protein. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular "PRO" polypeptide. These antibodies possess a "PRO"-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the "PRO" polypeptide and further binds tissue factor (TF).

#### vi. Heteroconjugate Antibodies

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange

reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

vii. Effector function engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.* **176**:1191-1195 (1992) and Shopes, B. *J. Immunol.* **148**:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* **53**:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design* **3**: 219-230 (1989).

viii. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof, or a small molecule toxin), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active protein toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, cholera toxin, botulinus toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcun, croton, *sapacnaria officinalis* inhibitor, gelonin, saporin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. Small molecule toxins include, for example, calicheamicins, maytansinoids, palytoxin and CC1065. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include  $^{212}\text{Bi}$ ,  $^{131}\text{I}$ ,  $^{131}\text{In}$ ,  $^{90}\text{Y}$  and  $^{186}\text{Re}$ .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis-(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* **238**: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient,

followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

#### ix. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.*, 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.*, 81(19)1484 (1989).

#### 10. Pharmaceutical Compositions

Antibodies specifically binding the product of an amplified gene identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of tumors, including cancers, in the form of pharmaceutical compositions.

If the protein encoded by the amplified gene is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, e.g. Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 7889-7893 [1993]).

Therapeutic formulations of the antibody are prepared for storage by mixing the antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as

sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Non-antibody compounds identified by the screening assays of the present invention can be formulated in an analogous manner, using standard techniques well known in the art.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

#### 11. Methods of Treatment

It is contemplated that the antibodies and other anti-tumor compounds of the present invention may be used to treat various conditions, including those characterized by overexpression and/or activation of the amplified genes identified herein. Exemplary conditions or disorders to be treated with such antibodies and other compounds, including, but not limited to, small organic and inorganic molecules, peptides, antisense

molecules, etc. include benign or malignant tumors (e.g. renal, liver, kidney, bladder, breast, gastric, ovarian, colorectal, prostate, pancreatic, ling, vulval, thyroid, hepatic carcinomas; sarcomas; glioblastomas; and various head and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and  
5 inflammatory, angiogenic and immunologic disorders.

The anti-tumor agents of the present invention, e.g. antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous administration of the  
10 antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-cancer agents, e.g. antibodies of the instant invention. For example, the patient to be treated with such anti-cancer agents may also receive radiation therapy. Alternatively, or in addition, a chemotherapeutic agent may be administered to the patient. Preparation and dosing schedules for such chemotherapeutic agents may be used according to  
15 manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the anti-tumor agent, e.g. antibody, or may be given simultaneously therewith. The antibody may be combined with an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP  
20 616812) in dosages known for such molecules.

It may be desirable to also administer antibodies against other tumor associated antigens, such as antibodies which bind to the ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial to also administer  
25 one or more cytokines to the patient. In a preferred embodiment, the antibodies herein are co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by an antibody of the present invention. However, simultaneous administration or administration of the antibody of the present invention first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory  
30 agent and the antibody herein.

For the prevention or treatment of disease, the appropriate dosage of an anti-tumor agent, e.g. an antibody herein will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent  
35 is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1-20mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations

over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

## 12. Articles of Manufacture

5 In another embodiment of the invention, an article of manufacture containing materials useful for the diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port  
10 (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually an anti-tumor agent capable of interfering with the activity of a gene product identified herein, e.g. an antibody. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice.

The article of manufacture may further comprise a second container comprising a pharmaceutically-  
15 acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

## 13. Diagnosis and Prognosis of Tumors

While cell surface proteins, such as growth receptors overexpressed in certain tumors are excellent  
20 targets for drug candidates or tumor (e.g. cancer) treatment, the same proteins along with secreted proteins encoded by the genes amplified in tumor cells find additional use in the diagnosis and prognosis of tumors. For example, antibodies directed against the proteins products of genes amplified in tumor cells can be used as tumor diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively  
25 detect the expression of proteins encoded by the amplified genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g. fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the amplified gene encodes a cell surface protein, e.g. a growth factor. Such binding assays are performed essentially as described in section 5 above.

30 *In situ* detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily  
35 available for *in situ* detection.

\*\*\*\*\*

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209. All original deposits referred to in the present application were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC § 122 and the Commissioner's rules pursuant thereto (including 37 CFR § 1.14 with particular reference to 886 OG 638).

Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as those described hereinabove and in the following textbooks: Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press N.Y., 1989; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., 1989; Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, inc., N.Y., 1990; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, 1988; Gait, M.J., *Oligonucleotide Synthesis*, IRL Press, Oxford, 1984; R.I. Freshney, *Animal Cell Culture* 1987; Coligan *et al.*, *Current Protocols in Immunology*, 1991.

### EXAMPLE 1

Isolation of cDNA clones Encoding Human PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882

#### 1. Isolation of cDNA clones encoding a human PRO201 (UNQ175) (nsp1)

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST was identified (1328938, DNA28710, Figure 29, SEQ ID NO: 29) which shared significant sequence identity with the adaptor protein Shc. Based upon the EST, a full-length cDNA clone (DNA30676, Nsp1, Figure 1, SEQ ID NO: 1) was obtained from a human fetal kidney library using an *in vivo* cloning technique. DNA30676 has a single long open reading frame which encodes a 576 amino acids protein (Figure 2, SEQ ID NO: 2). A vector containing DNA30676 (DNA30676-1223) was deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, on December 23, 1998 and assigned ATCC Deposit No. 209567.

#### 2. Isolation of cDNA clones encoding a human PRO292 (UNQ266) (Cathepsin-d)

PRO292 is identical with the human death associated protein DAP-7, also called cathepsin D. The amino acid sequence of this 412 amino acids protein is present in the public Dayhoff database under Accession Nos. CATD\_Human and P\_R74207, and is shown in Figure 4 (SEQ ID NO:4). The nucleotide sequence of the DNA encoding PRO292 is shown in Figure 3 (SEQ ID NO: 3). DAP-7 has also been disclosed in WO 95/10630 published on April 20, 1995, and in Faust *et al.*, *Proc. Natl. Acad. Sci. USA* **82**: 4910-4914 (1985).

### 3. Isolation of cDNA clones encoding a human PRO327 (UNO288)

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and various EST sequences were identified that showed certain homology to human prolactin receptor protein. Based upon a consensus sequence extended using repeated cycles of BLAST and the program "phrap" (Phil Green, University of Washington, Seattle, Washington), oligonucleotides were synthesized and a cDNA clone designated DNA38113 (Figure 5, SEQ ID NO: 5) was isolated from a human fetal lung tissue library (LIB26) and sequenced. Clone DNA38113 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 119-121, and ending at the stop codon at nucleotide positions 1385-1387 (Figure 5). The predicted polypeptide precursor, PRO327, is 422 amino acids long (Figure 6, SEQ ID NO: 6). The full-length PRO327 protein has an estimated molecular weight of about 46.302 daltons and a pI of about 9.42.

A vector containing DNA38113 (DNA38113-1230) was deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209, on December 10, 1997 and assigned ATCC Deposit No. 209530.

### 4. Isolation of cDNA clones encoding human PRO1265 (UNO636)

DNA60764 was identified by applying a proprietary signal sequence finding algorithm developed by Genentech, Inc. (South San Francisco, CA) upon ESTs as well as clustered and assembled EST fragments from public (e.g., GenBank) and/or private (LIFESEQ®, Incyte Pharmaceuticals, Inc., Palo Alto, CA) databases. The signal sequence algorithm computes a secretion signal score based on the character of the DNA nucleotides surrounding the first and optionally the second methionine codon(s) (ATG) at the 5'-end of the sequence or sequence fragment under consideration. The nucleotides following the first ATG must code for at least 35 unambiguous amino acids without any stop codons. If the first ATG has the required amino acids, the second is not examined. If neither meets the requirement, the candidate sequence is not scored. In order to determine whether the EST sequence contains an authentic signal sequence, the DNA and corresponding amino acid sequences surrounding the ATG codon are scored using a set of seven sensors (evaluation parameters) known to be associated with secretion signals.

Use of the above described signal sequence algorithm allowed identification of an EST cluster sequence from the LIFESEQ® database, designated EST Cluster No. 86995 that was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* **266**:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into a



consensus DNA sequence with the program "phrap". One or more of the ESTs used in the assembly was derived from a cDNA library prepared from RNA isolated from inflamed human adenoid tissue. A consensus sequence was assembled, and, based on homology between the consensus sequence and Incyte EST no. 20965 shown in Figure 26 (SEQ ID NO: 26), EST clone no. 20965 was purchased and the cDNA insert was obtained and sequenced. The sequence of this cDNA insert is shown in Figure 7 (SEQ ID NO: 7) is herein designated as DNA60764.

The full length clone shown in Figure 7 contained a single open reading frame with an apparent translational initiation site at nucleotide positions 79-81 and ending at the stop codon found at nucleotide positions 1780-1782 (Figure 7, SEQ ID NO: 7). The predicted polypeptide precursor (Figure 8, SEQ ID NO: 8) is 567 amino acids long. PRO1265 has a calculated molecular weight of approximately 62,881 daltons and an estimated pI of approximately 8.97. Additional features include a signal peptide sequence at about amino acids 1-21; potential N-glycosylation sites at about amino acids 54-57, 134-137, 220-223, and 559-562; and a region having amino acid sequence identity with D-amino acid oxidase proteins at about amino acids 61-80.

Clone DNA60764 (UNQ636), designated as DNA60764-1533 was deposited with the ATCC on November 10, 1998, and is assigned ATCC deposit no. 203452.

#### 5. Isolation of cDNA clones encoding human PRO344 (UNQ303)

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). This method will be hereinafter referred to as "ECD homology search".

A consensus DNA sequence was assembled relative to other EST sequences using phrap. The consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the sequence as far as possible using the sources of EST sequences discussed above. Using probes synthesized based upon the extended consensus sequence, a cDNA clone designated DNA40592 was isolated from a human fetal kidney tissue library.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO344 [herein designated as UNQ303 (DNA40592)] (Figure 9, SEQ ID NO:9) and the derived protein sequence for PRO344 (Figure 10, SEQ ID NO: 10). Clone UNQ303 (DNA40592) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 227-229 and ending at the stop codon at nucleotide positions 956-958 (Figure 9). The predicted polypeptide precursor is 243 amino acids long (Figure 10). Important regions of the amino acid sequence encoded by nucleotides 1 to 729 of PRO344 include the signal peptide, corresponding to amino acids 1-15, the start of the mature protein, corresponding to amino acids 16-67, and two potential N-myristoylation sites, corresponding to amino acids 68-215 and 216-

243, respectively. Clone DNA40592 (DNA40592-1242) was deposited with ATCC on November 21, 1997 and assigned ATCC deposit No.209492.

6. Isolation of cDNA clones encoding human PRO343

Following the ECD homology search described in section 5 above, a consensus sequence was identified. Based on this sequence, oligonucleotides were synthesized and used to isolate cDNA clones from a human fetal lung tissue (library LIB26).

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO343 [herein designated as UNQ302 (DNA43318)] (Figure 11, SEQ ID NO:11), and the derived protein sequence for PRO343.

The entire nucleotide sequence of UNQ302 (DNA43318) is shown in Figure 11 (SEQ ID NO:11). Clone UNQ302 (DNA43318) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 53-55 and ending at the stop codon at nucleotide positions 1004-1006 (Figure 11). The predicted polypeptide precursor is 317 amino acids long (Figure 12). Various unique aspects of the PRO343 protein are shown in Figure 12. Clone UNQ302 (DNA43318-1217) was deposited with ATCC on November 21, 1997, and is assigned ATCC deposit no. 209481.

7. Isolation of cDNA clones encoding human PRO347

Using the ECD homology search described above, a consensus sequence was determined. Based on the consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO347. RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB228). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO347 [herein designated as UNQ306 (DNA44176)] (Figure 13, SEQ ID NO:13) and the derived protein sequence for PRO347 (Figure 14, SEQ ID NO: 14).

The entire nucleotide sequence of UNQ306 (DNA44176) is shown in Figure 13 (SEQ ID NO:13). Clone UNQ306 (DNA44176) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 123-125 and ending at the stop codon at nucleotide positions 1488-1490 (Figure 13). The predicted polypeptide precursor is 455 amino acids long (Figure 14, SEQ ID NO: 14). The full-length PRO347 protein shown in Figure 14 has an estimated molecular weight of about 50,478 daltons and a pI of about 8.44. Clone UNQ306 (DNA44176) was deposited with ATCC on December 10, 1997, and is assigned ATCC deposit no. 209532.

8. Isolation of cDNA clones encoding human PRO357

The sequence expression tag "2452972" (Figure 30, SEQ ID NO: 30) by Incyte Pharmaceuticals, Palo Alto, CA, was used to begin a data base search. The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases which overlapped with a portion of "2452972". The EST databases included public EST databases (e.g., GenBank) and a proprietary EST DNA

database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed as described above for ECD homology searches.

A consensus DNA sequence was assembled relative to other EST sequences using phrap. Using probes synthesized based upon the consensus sequence, a cDNA clone designated DNA44804 was isolated from a human fetal liver library, and sequenced in its entirety. The entire nucleotide sequence of DNA44804 is shown in Figure 15 (SEQ ID NO: 15). DNA44804 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 137-139 and ending at the stop codon at nucleotide positions 1931-1933. The predicted polypeptide precursor is 598 amino acids long (Figure 16, SEQ ID NO: 16). Clone DNA44804-1248 was deposited with ATCC on December 10, 1997 and is assigned ATCC deposit No. 209527.

#### 9. Isolation of cDNA clones encoding PRO715

A proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched for EST sequences encoding polypeptides having homology to human TNF- $\alpha$ . This search resulted in the identification of Incyte Expressed Sequence Tag No. 2099855 (see Figure 31, SEQ ID NO: 31). Based upon the alignment of various EST clones, a single clone (725887, Accession No. AA292358) was sequenced. The full-length DNA52722 sequence was then obtained from sequencing the insert DNA from clone 725887 (Accession No. AA292358).

The entire nucleotide sequence of DNA52722 is shown in Figure 17 (SEQ ID NO: 17). Clone DNA52722 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 114-116 and ending at the stop codon at nucleotide positions 864-866 (Figure 17). The predicted polypeptide is 250 amino acids long (Figure 18, SEQ ID NO: 18). The full-length PRO715 protein shown in Figure 18 has an estimated molecular weight of about 27,433 daltons and a pI of about 9.85.

Analysis of the amino acid sequence of the full-length PRO715 polypeptide suggests that it possesses significant homology to members of the tumor necrosis factor family of proteins, thereby indicating that PRO715 is a novel tumor necrosis factor protein.

A vector containing DNA52722 (DNA52722-1229) was deposited with ATCC on January 7, 1998 and was assigned ATCC deposit no. 209570.

#### 10. Isolation of cDNA clones encoding PRO1017

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank, Merck/Wash. U.) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altschul *et al.*, *Methods in Enzymology* 266: 460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

An initial consensus DNA sequence was assembled relative to other EST sequences using phrap. The initial consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. An extended consensus sequence was then performed using the same procedures.

- 5 Further examination of the two consensus assemblies generated in the previous paragraph. EST clone AA243086 (Merck clone 664402) was further examined. DNA sequencing gave the full-length DNA sequence for PRO1017 [herein designated as UNQ500 (DNA56112-1379)] (SEQ ID NO: 19) and the derived protein sequence for PRO1017.

- The entire nucleotide sequence of DNA56112 is shown in Figure 19 (SEQ ID NO: 19). Clone  
10 UNQ500 (DNA56112-1379) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 128-130 and ending at the stop codon at nucleotide positions 1370-1372. The predicted polypeptide precursor is 414 amino acids long Figure 20. The full-length PRO1017 protein in Figure 20 has an estimated molecular weight of about 48414 daltons and a pI of about 9.54. Clone DNA56112 has been deposited with the ATCC, and has been assigned deposit number 209883. In the event of a conflict between  
15 the sequence of the cDNA insert of the deposit and the sequence described herein, it is understood that the deposit contains the correct sequence.

#### 11. Isolation of cDNA clones encoding PRO1112

- The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were used to search expressed sequence  
20 tag (EST) databases, following the ECD homology search described above. The EST databases included public EST databases (e.g., GenBank, Merck/Wash. U) and a proprietary EST DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA).

The clustering and assembling of the public and private ESTs into one or several consensus sequences to create a candidate sequence was performed using repeated cycles of the computer program phrap.

- 25 Candidate sequences with a sufficient score were further examined with the computer program blast or blast-2 (Altschul, SF, and W Gish (1996), Local alignment statistics. ed. R. Doolittle, *Methods in Enzymology* 266: 460-480) to confirm the novelty of the sequence. A candidate or initial consensus sequence was formed from sequences identified in an Incyte database which fit the signal algorithm.

- Based on the discoveries and information provided herein, Merck EST AA223646, clone 650953,  
30 from library 318, a human neuroepithelium tissue library, was further examined. DNA sequencing of the clone gave UNQ555 (DNA57702-1476) (Figure 21, SEQ ID NO:21), which includes the full-length DNA sequence for a PRO1112.

- The entire nucleotide sequence of UNQ555 (DNA57702-1476) is shown in Figure 21 (SEQ ID NO:21). Clone UNQ555 (DNA57702-1476) contains a single open reading frame with an apparent  
35 translational initiation site at nucleotide positions 20-22 and ending at the stop codon at nucleotide positions 806-808 of SEQ ID NO:21 (Figure 21). The predicted polypeptide precursor is 262 amino acids long (Figure 22, SEQ ID NO: 22). The full-length PRO1112 protein shown in Figure 22 has an estimated molecular weight of about 29379 daltons and a pI of about 8.93. Figure 22 also shows the approximate locations of the signal peptide and transmembrane domains. Clone UNQ555 (DNA57702-1476) was deposited with the ATCC on

9 June 1998, and assigned ATCC Deposit No. 209951.

12. Isolation of cDNA clones encoding human PRO509

To isolate a cDNA for HVEM, a bacteriophage library of human retinal cDNA (commercially available from Clontech) was screened by hybridization with a synthetic oligonucleotide probe based on an EST sequence (GenBank locus AA021617), which showed some degree of homology to members of the TNFR family. Five positive clones (containing cDNA inserts of 1.8-1.9kb) were identified in the cDNA library, and the positive clones were confirmed to be specific by PCR using the above hybridization probe as a PCR primer. Single phage plaques containing each of the five positive clones were isolated by limiting dilution and the DNA was purified using a Wizard Lambda Prep DNA purification kit (commercially available from Promega).

The cDNA inserts from three of the five bacteriophage clones were excised from the vector arms by digestion with EcoRI, gel-purified, and subcloned into pRK5 and sequenced on both strands. The three clones contained an identical open reading frame (with the exception of an intron found in one of the clones).

The entire nucleotide sequence of HVEM (DNA50148) is shown in Figure 23 (SEQ ID NO: 23). The cDNA contained one open reading frame with a translational initiation site assigned to the ATG codon at nucleotide positions 86-88. The surrounding sequence at this site is in reasonable agreement with the proposed consensus sequence for initiation sites [Kozak, *J. Cell. Biol.*, **115**: 887-903 (1991)]. The open reading frame ends at the termination codon TGA at nucleotide positions 925-927.

The predicted amino acid sequence of the full length HVEM (UNQ329) contains 283 amino acids (See Fig. 24, SEQ ID NO: 24). A putative transmembrane region of the HVEM comprises amino acids 201-225 of Fig. 24 and a putative cytoplasmic region of the HVEM comprises amino acids 226-283 of Fig. 24. The sequence differs from the HVEM sequence reported in Montgomery et al., *supra*, in at least two amino acids: as shown in Fig. 26, codon 108 encodes a serine and codon 140 encodes an alanine. An alignment (using the Align™ computer program) of a 58 amino acid long cytoplasmic region of HVEM with other known members of the human TNF receptor family showed some sequence similarity, in particular to CD40 (12 identities) and LT-beta receptor (11 identities).

13. Isolation of cDNA clones encoding PRO853

Using the ECD homology search described above, a consensus sequence was determined. Based on the consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for the PRO853 sequence (UNQ419). RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue (LIB228). The cDNA libraries used to isolate the cDNA clones were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for a PRO853 [herein designated as DNA48227] (Figure 25, SEQ ID NO: 25) and the derived PRO853 protein (UNQ419) (Figure 26, SEQ ID NO: 26).

The entire nucleotide sequence of DNA48227 is shown in Figure 25 (SEQ ID NO: 25). Clone DNA48227-1350 contains a single open reading frame with an apparent translational initiation site at

nucleotide positions 128-130 and ending at the stop codon at nucleotide positions 1259-1261 (Figure 25). The predicted polypeptide precursor is 377 amino acids long (Figure 26)(SEQ ID NO: 26). The full-length PRO853 (UNQ419) protein shown in Figure 26 (SEQ ID NO: 26) has an estimated molecular weight of about 40,849 daltons and a pI of about 7.98. Important regions of the amino acid sequence of UNQ419 include the  
 5 signal peptide, corresponding to amino acids from about 1 to about 16 of SEQ ID NO: 26, the glycosaminoglycan attachment site, corresponding to amino acids from about 46 to about 49 of SEQ ID NO: 26, and two sequences typical of the short-chain alcohol dehydrogenase family, corresponding to amino acids from about 37 to about 49 and about 114 to about 124 of SEQ ID NO: 26, respectively. Clone DNA48227-1350 has been deposited with the ATCC and is assigned ATCC deposit no. 209812.

10 14. Isolation of cDNA clones encoding human PRO882

PRO882 (UNQ448) is identical with cardiotrophin-1. The amino acid sequence of this 201 amino acid protein is present in the public Dayhoff database under Accession Nos. P\_R83967, P\_W29238 and CTF1\_HUMAN, among others, and is shown in Figure 28. The nucleotide of the DNA encoding the PRO882 (UNQ448) is shown in Figure 29 (SEQ ID NO: 29). Cardiotrophin-1 has also been disclosed in WO9730146,  
 15 published on 21 August 1997 and WO9529237, published on 2 November 1995.

EXAMPLE 2

Gene Amplification

This example shows that the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding genes are  
 20 amplified in the genome of certain human lung, colon and/or breast cancers and/or cell lines. Amplification is associated with overexpression of the gene product, indicating that the binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 proteins are useful targets for therapeutic intervention in certain cancers such as colon, lung, breast  
 25 and other cancers. Therapeutic agent may take the form of antagonists of binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding genes, for example, murine-human chimeric, humanized or human antibodies against a binding specificities for at least two different antigens. In the present case, one of the binding specificities  
 30 is for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.

The starting material for the screen was genomic DNA isolated from a variety cancers. The DNA is quantitated precisely, e.g. fluorometrically. As a negative control, DNA was isolated from the cells of ten normal healthy individuals which was pooled and used as assay controls for the gene copy in healthy  
 35 individuals (not shown). The 5' nuclease assay (for example, TaqMan™) and real-time quantitative PCR (for example, ABI Prizm 7700 Sequence Detection System™ (Perkin Elmer, Applied Biosystems Division, Foster City, CA)), were used to find genes potentially amplified in certain cancers. The results were used to

determine whether the DNA encoding binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 is over-represented in any of the primary lung or colon cancers or cancer cell lines or breast cancer cell lines that were screened. The primary lung cancers were obtained from individuals with tumors of the type and stage as indicated in Table 1. An explanation of the abbreviations used for the designation of the primary tumors listed in Table 1 and the primary tumors and cell lines referred to throughout this example has been given hereinbefore.

The results of the Taqman™ are reported in delta ( $\Delta$ ) CT units. One unit corresponds 1 PCR cycle or approximately a 2-fold amplification relative to normal, two units corresponds to 4-fold, 3 units to 8-fold amplification and so on. Quantitation was obtained using primers and a Taqman™ fluorescent probe derived from the binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201-, PRO292-, PRO327-, PRO1265-, PRO344-, PRO343-, PRO347-, PRO357-, PRO715-, PRO1017-, PRO1112-, PRO509-, PRO853- or PRO882-encoding gene. Regions of binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 which are most likely to contain unique nucleic acid sequences and which are least likely to have spliced out introns are preferred for the primer and probe derivation, e.g. 3-untranslated region. The sequences for the primers and probes (forward, reverse and probe) used for the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 gene amplification were as follows:

PRO201 (DNA30676):

30676.tm.f

5'-CGCAGACACCCTTCTTCACA-3' (SEQ ID NO: 32)

30676.tm.r

25 5'-CGACTCCTTTGGTCTCTTCTGG-3' (SEQ ID NO: 33)

30676.tm.p

5'-CCGGGACCCCCAGGTTTTTGC-3' (SEQ ID NO: 34)

PRO292 (DNA35617):

35617.tm.f

30 5'-GATCCTGGG CGACGTCTTC-3' (SEQ ID NO: 35)

35617.tm.p

5'-TCGGCCGCTACTACACTGTGTTTGACC-3' (SEQ ID NO: 36)

35617.tm.r

5'-GCCCCACCCTGTTGTTGTCA-3' (SEQ ID NO: 37)

35 PRO327 (DNA38113):

38113.tm.f

5'-CTCAAGAAGCACG CGTACTGC-3' (SEQ ID NO: 38)

38113.tm.p

5'-CCAACCTCAGCTTCCGCCTCTACGA-3' (SEQ ID NO: 39)

38113.tm.r

5'-CATCCAGGCTCGCCACTG-3' (SEQ ID NO: 40)

5 PRO1265 (DNA60764):

60764.tm.fl

5'-TGACCTGGCAAAGGAAGAA-3' (SEQ ID NO: 41)

60764.tm.pl

5'-CAGCCACCCTCCAGTCCAAGG-3' (SEQ ID NO: 42)

10 60764.tm.rl

5'-GGGTCGTGTTTTGGAGAGA-3' (SEQ ID NO: 43)

PRO344 (DNA40592):

40592.tm.fl

5'-TGGCAAGGAATGGGAACAGT-3' (SEQ ID NO: 44)

15 40592.tm.pl

5'-ATGCTGC CAGACCTGAT CGCAGACA-3' (SEQ ID NO: 45)

40592.tm.rl

5'-G GGCAGAAATC CAGCCACT-3' (SEQ ID NO: 46)

PRO343 (DNA43318):

20 43318.tm.fl

5'-TCTACATCAGCCTCTCTGCGC-3' (SEQ ID NO: 47)

43318.tm.pl

5'-CGATCTTCTCCACCCAGGAGCGG-3' (SEQ ID NO: 48)

43318.tm.rl

25 5'-GGAGCTGCACCCCTTGC-3' (SEQ ID NO: 49)

PRO347 (DNA44176):

44176.tm.fl

5'-CCCTTCGCCTGCTTTTGA-3' (SEQ ID NO: 50)

44176.tm.pl

30 5'-GCCATCTAATTGAAGCCCATCTTCCA-3' (SEQ ID NO: 51)

44176.tm.rl

5'-CTGGCGGTGT CCTTCCTT-3' (SEQ ID NO: 52)

PRO357 (DNA44804):

44804.tm.fl



5'-CCTCGGTCTCCTCATCTGTGA-3' (SEQ ID NO: 53)

44804.tm.pl

5'-TGGCCCAGCTGACGAGCCCT-3' (SEQ ID NO: 54)

44804.tm.rl

5 5'-CTCATAGGCACTCGGTTCTGG-3' (SEQ ID NO: 55)

PRO715 (DNA52722):

52722.tm.fl

5'-TGGCTCCCAGCTTGGAAGA-3' (SEQ ID NO: 56)

52722.tm.pl

10 5'-CAGCTCTTGGCTGTCTCCAGTATGTACCCA-3' (SEQ ID NO: 57)

52722.tm.rl

5'-GATGCCTCTGTTCTGCACAT-3' (SEQ ID NO: 58)

PRO1017 (DNA56112):

15 56112.tm.fl

5'-CCTCCTCCGAGACTGAAAGCT-3' (SEQ ID NO: 59)

56112.tm.pl

5'-TCGCGTTGCTTTTTCTCGCGTG-3' (SEQ ID NO: 60)

56112.tm.rl

20 5'-GCGTGCATC AGGTTCCA-3' (SEQ ID NO: 61)

PRO1112 (DNA57702):

57702.tm.fl

5'-GTCCCTTCACTGTTTAGAGCATGA-3' (SEQ ID NO: 62)

57702.tm.pl

25 5'-ACTCTCCCCCTCAACAGCCTCCTGAG-3' (SEQ ID NO: 63)

57702.tm.rl

5'-GTGG TCAGGGCAGA TCCTTT-3' (SEQ ID NO: 64)

PRO509 (DNA50148):

50148.tm.fl

30 5'-GGAGGAGACAATACCCTCATTCA-3' (SEQ ID NO: 65)

50148.tm.pl

5'-AGCAGCCGTCGCTCCAGGTATCTC-3' (SEQ ID NO: 66)

50148.tm.rl

5'-CCA GGTGGACAGCCTCTTTC-3' (SEQ ID NO: 67)

35 PRO853 (DNA48227)

48227.tm.fl

5'-GGCACTTCATGGTCCTTGAAA-3' (SEQ ID NO: 68)

48227.tm.pl

5'-CGGATGTGTGTGAGGCCATGCC-3' (SEQ ID NO: 69)

5 48227.tm.rl

5'-GAAAGTA ACCACGGAGG TCAAGAT-3' (SEQ ID NO: 70)

PRO882 (DNA58125)

58125.tm.fl

5'-TTCCCAGCCTCTCTTTGCTTT-3' (SEQ ID NO: 71)

10 58125.tm.pl

5'-TGCCCCGTTCTCTTA ACTCTTGGACCC-3' (SEQ ID NO: 72)

58125.tm.rl

5'-TCAGACGGAGTTACCATGCAGA-3' (SEQ ID NO: 73)

The 5' nuclease assay reaction is a fluorescent PCR-based technique which makes use of the 5' exonuclease activity of Taq DNA polymerase enzyme to monitor amplification in real time. Two oligonucleotide primers are used to generate an amplicon typical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the TAQ DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

The 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI Prism 7700TM Sequence Detection. The system consists of a thermocycler, laser, charge-coupled device (CCD) camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

5' Nuclease assay data are initially expressed as Ct, or the threshold cycle. This is defined as the cycle at which the reporter signal accumulates above the background level of fluorescence. The  $\Delta C_t$  values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing cancer DNA results to normal human DNA results.

Table 1 describes the stage, T stage and N stage of various primary tumors which were used to screen the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 compounds of the invention.

Table 1  
Primary Lung and Colon Tumor Profiles

	Primary Tumor	Stage	Other Stage	Dukes Stage	T Stage	N Stage
5	Human lung tumor SqCCA (SRCC724) [LT1]	IB	--	--	T1	N1
	Human lung tumor NSCCa (SRCC725) [LT1a]	IA	--	--	T3	N0
	Human lung tumor AdenoCa (SRCC726) [LT2]	IB	--	--	T2	N0
	Human lung tumor AdenoCa (SRCC727) [LT3]	IB	--	--	T1	N2
	Human lung tumor SqCCq (SRCC728) [LT4]	IIB	--	--	T2	N0
10	Human lung tumor AdenoCa (SRCC729) [LT6]	IV	--	--	T1	N0
	Human lung tumor Aden/SqCCa (SRCC730) [LT7]	IB	--	--	T1	N0
	Human lung tumor AdenoCa (SRCC731) [LT9]	IIB	--	--	T2	N0
	Human lung tumor SqCCa (SRCC732) [LT10]	IA	--	--	T2	N1
	Human lung tumor AdenoCa (SRCC733) [LT11]	IB	--	--	T1	N1
15	Human lung tumor AdenoCa (SRCC734) [LT12]	IIA	--	--	T2	N0
	Human lung tumor BAC (SRCC735) [LT13]	IB	--	--	T2	N0
	Human lung tumor SqCCa (SRCC736) [LT15]	IB	--	--	T2	N0
	Human lung tumor SqCCa (SRCC737) [LT16]	IB	--	--	T2	N0
	Human lung tumor SqCCa (SRCC738) [LT17]	IIB	--	--	T2	N1
20	Human lung tumor SqCCa (SRCC739) [LT18]	IB	--	--	T2	N0
	Human lung tumor SqCCa (SRCC740) [LT19]	IB	--	--	T2	N0
	Human lung tumor LCCa (SRCC741) [LT21]	IIB	--	--	T3	N1
	Human colon AdenoCa (SRCC742) [CT2]	--	M1	D	pT4	N0
	Human colon AdenoCa (SRCC743) [CT3]		--	B	pT3	N0
25	Human colon AdenoCa (SRCC 744) [CT8]			B	T3	N0

	Human colon AdenoCa (SRCC745) [CT10]			A	pT2	N0
	Human colon AdenoCa (SRCC746) [CT12]		MO, R1	B	T3	N0
	Human colon AdenoCa (SRCC747) [CT14]		pMO, RO	B	pT3	pN0
	Human colon AdenoCa (SRCC748) [CT15]		M1, R2	D	T4	N2
5	Human colon AdenoCa (SRCC749) [CT16]		pMO	B	pT3	pN0
	Human colon AdenoCa (SRCC750) [CT17]			C1	pT3	pN1
	Human colon AdenoCa (SRCC751) [CT1]		MO, R1	B	pT3	N0
	Human colon AdenoCa (SRCC752) [CT4]			B	pT3	M0
	Human colon AdenoCa (SRCC753) [CT5]		G2	C1	pT3	pN0
10	Human colon AdenoCa (SRCC754) [CT6]		pMO, RO	B	pT3	pN0
	Human colon AdenoCa (SRCC755) [CT7]		G1	A	pT2	pN0
	Human colon AdenoCa (SRCC756) [CT9]		G3	D	pT4	pN2
	Human colon AdenoCa (SRCC757) [CT11]			B	T3	N0
i5	Human colon AdenoCa (SRCC758) [CT18]		MO, RO	B	pT3	pN0

#### DNA Preparation:

DNA was prepared from cultured cell lines, primary tumors, normal human blood. The isolation was performed using purification kit, buffer set and protease and all from Quiagen. according to the manufacturer's instructions and the description below.

20

#### *Cell culture lysis:*

Cells were washed and trypsinized at a concentration of  $7.5 \times 10^8$  per tip and pelleted by centrifuging at 1000 rpm for 5 minutes at 4°C, followed by washing again with 1/2 volume of PBS recentrifugation. The pellets were washed a third time, the suspended cells collected and washed 2x with PBS. The cells were then suspended into 10 mL PBS. Buffer C1 was equilibrated at 4°C. Quiagen protease #19155 was diluted into 25 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and equilibrated at 4°C. 10 mL of G2 Buffer was prepared by diluting Quiagen RNase A stock (100 mg/ml) to a final concentration of 200 µg/ml.

Buffer C1 (10 mL, 4°C) and ddH<sub>2</sub>O (40 mL, 4°C) were then added to the 10 mL of cell suspension, mixed by inverting and incubated on ice for 10 minutes. The cell nuclei were pelleted by centrifuging in a Beckman swinging bucket rotor at 2500 rpm at 4°C for 15 minutes. The supernatant was discarded and the 30 nuclei were suspended with a vortex into 2 mL Buffer C1 (at 4°C) and 6 mL ddH<sub>2</sub>O, followed by a second 4°C centrifugation at 2500 rpm for 15 minutes. The nuclei were then resuspended into the residual buffer using 200 µl per tip. G2 buffer (10 ml) was added to the suspended nuclei while gentle vortexing was applied. Upon completion of buffer addition, vigorous vortexing was applied for 30 seconds. Quiagen protease (200 µl, prepared as indicated above) was added and incubated at 50°C for 60 minutes. The incubation and 35 centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting

at 3000 x g for 10 min., 4°C).

*Solid human tumor sample preparation and lysis:*

Tumor samples were weighed and placed into 50 ml conical tubes and held on ice. Processing was limited to no more than 250 mg tissue per preparation (1 tip/preparation). The protease solution was freshly prepared by diluting into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer (20 ml) was prepared by diluting DNase A to a final concentration of 200 mg/ml (from 100 mg/ml stock). The tumor tissue was homogenated in 19 ml G2 buffer for 60 seconds using the large tip of the polytron in a laminar-flow TC hood to order to avoid inhalation of aerosols, and held at room temperature. Between samples, the polytron was cleaned by spinning at 2 x 30 seconds each in 2L ddH<sub>2</sub>O, followed by G2 buffer (50 ml). If tissue was still present on the generator tip, the apparatus was disassembled and cleaned.

Quiagen protease (prepared as indicated above, 1.0 ml) was added, followed by vortexing and incubation at 50°C for 3 hours. The incubation and centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Human blood preparation and lysis:*

Blood was drawn from healthy volunteers using standard infectious agent protocols and citrated into 10 ml samples per tip. Quiagen protease was freshly prepared by dilution into 6.25 ml cold ddH<sub>2</sub>O to a final concentration of 20 mg/ml and stored at 4°C. G2 buffer was prepared by diluting RNase A to a final concentration of 200 µg/ml from 100 mg/ml stock. The blood (10 ml) was placed into a 50 ml conical tube and 10 ml C1 buffer and 30 ml ddH<sub>2</sub>O (both previously equilibrated to 4°C) were added, and the components mixed by inverting and held on ice for 10 minutes. The nuclei were pelleted with a Beckman swinging bucket rotor at 2500 rpm, 4°C for 15 minutes and the supernatant discarded. With a vortex, the nuclei were suspended into 2 ml C1 buffer (4°C) and 6 ml ddH<sub>2</sub>O (4°C). Vortexing was repeated until the pellet was white. The nuclei were then suspended into the residual buffer using a 200 µl tip. G2 buffer (10 ml) were added to the suspended nuclei while gently vortexing, followed by vigorous vortexing for 30 seconds. Quiagen protease was added (200 µl) and incubated at 50°C for 60 minutes. The incubation and centrifugation was repeated until the lysates were clear (e.g., incubating additional 30-60 minutes, pelleting at 3000 x g for 10 min., 4°C).

*Purification of cleared lysates:*

(1) Isolation of genomic DNA:

Genomic DNA was equilibrated (1 sample per maxi tip preparation) with 10 ml QBT buffer. QF elution buffer was equilibrated at 50°C. The samples were vortexed for 30 seconds, then loaded onto equilibrated tips and drained by gravity. The tips were washed with 2 x 15 ml QC buffer. The DNA was eluted into 30 ml silanized, autoclaved 30 ml Corex tubes with 15 ml QF buffer (50°C). Isopropanol (10.5 ml) was added to each sample, the tubes covered with parafin and mixed by repeated inversion until the DNA precipitated. Samples were pelleted by centrifugation in the SS-34 rotor at 15,000 rpm for 10 minutes at 4°C. The pellet location was marked, the supernatant discarded, and 10 ml 70% ethanol (4°C) was added. Samples were pelleted again by centrifugation on the SS-34 rotor at 10,000 rpm for 10 minutes at 4°C. The pellet location was marked and the supernatant discarded. The tubes were then placed on their side in a drying rack and dried 10 minutes at 37°C, taking care not to overdry the samples.

After drying, the pellets were dissolved into 1.0 ml TE (pH 8.5) and placed at 50°C for 1-2 hours. Samples were held overnight at 4°C as dissolution continued. The DNA solution was then transferred to 1.5 ml tubes with a 26 gauge needle on a tuberculin syringe. The transfer was repeated 5x in order to shear the DNA. Samples were then placed at 50°C for 1-2 hours.

5           (2)     Quantitation of genomic DNA and preparation for gene amplification assay:

The DNA levels in each tube were quantified by standard A260, A280 spectrophotometry on a 1:20 dilution (5 µl DNA + 95 µl ddH<sub>2</sub>O) using the 0.1 ml quartz cuvetts in the Beckman DU640 spectrophotometer. A260/A280 ratios were in the range of 1.8-1.9. Each DNA samples was then diluted further to approximately 200 ng/ml in TE (pH 8.5). If the original material was highly concentrated (about 700 ng/µl), the material was placed at 50°C for several hours until resuspended.

10           Fluorometric DNA quantitation was then performed on the diluted material (20-600 ng/ml) using the manufacturer's guidelines as modified below. This was accomplished by allowing a Hoeffer DyNA Quant 200 fluorometer to warm-up for about 15 minutes. The Hoechst dye working solution (#H33258, 10 µl, prepared within 12 hours of use) was diluted into 100 ml 1 x TNE buffer. A 2 ml cuvette was filled with the  
15           fluorometer solution, placed into the machine, and the machine was zeroed. pGEM 3Zf(+) (2 µl, lot #360851026) was added to 2 ml of fluorometer solution and calibrated at 200 units. An additional 2 µl of pGEM 3Zf(+) DNA was then tested and the reading confirmed at 400 +/- 10 units. Each sample was then read at least in triplicate. When 3 samples were found to be within 10% of each other, their average was taken and this value was used as the quantification value.

20           The fluorometricly determined concentration was then used to dilute each sample to 10 ng/µl in ddH<sub>2</sub>O. This was done simultaneously on all template samples for a single TaqMan plate assay, and with enough material to run 500-1000 assays. The samples were tested in triplicate with Taqman™ primers and probe both B-actin and GAPDH on a single plate with normal human DNA and no-template controls. The diluted samples were used provided that the CT value of normal human DNA subtracted from test DNA was  
25           +/- 1 CT. The diluted, lot-qualified genomic DNA was stored in 1.0 ml aliquots at -80°C. Aliquots which were subsequently to be used in the gene amplification assay were stored at 4°C. Each 1 ml aliquot is enough for 8-9 plates or 64 tests.

*Gene amplification assay:*

30           The PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 compounds of the invention were screened in the following primary tumors and the resulting ΔCt values are reported in Table 2.

Table 2  
 $\Delta$ Ct values in lung and colon primary tumor and cell line models

Primary Tumor	PRO201	PRO292	PRO327	PRO1265	PRO344	PRO343	PRO347	PRO357	PRO715	PRO1017	PRO1112	PRO509	PRO853	PRO882
SRC724 (LT1)	0.27,	1.385,	0.045,	-1.555	-0.035,	-0.5,	1.035, 0.1,	-0.39,	0.355,	0.805	-0.005	1.115,	0.18,	0.45,
	0.05,	-1.775,	-0.155		-0.435	-1.16,	-0.71,	-0.16,	1.625,			0.79,	0.23	0.685
	0.41,	-1.115,			-1.07,	-1.07,	-0.14,	0.73,	0.07			-0.235,		
	-0.27	-1.29			-1.16	-1.16	-0.53	0.38,				0.185		
SRC725 (LT1a)	1.15,	1.435,	1.045,	0.005	0.815,	-0.3,	1.865, 1,	1.18,	1.045,	1.785	0.815	1.135,	0.98,	1.31,
	0.99,	-1.025,	0.385		0.645	-0.16,	0.63, 1.93,	2.47,	-0.385,			0.85,	0.29	1.045
	-0.1,	0.035,			-0.56	-0.56	0.87	0.9,	0.61			-0.725,		
	0.04	-0.02			-1.76	-1.76		0.58,				-0.395		
SRC726 (LT2)	0.22,	-0.785,	-0.255,	-0.355	0.025,	-1.23,	-0.315,	-0.91,	0.225,	0.855	0.125	0.515,	0.19,	0.18,
	0.24,	-1.365,	0.465		0.425	-0.25,	-0.69,	-0.44,	0.365,			0.45,	0.78	0.435
	-0.1,	-0.145,				-0.58,	-0.69,	0.23,	0.15			-0.055,		
	0.04	-0.24				-0.57	0.07,-2.26	0.13,				0.315		
SRC727 (LT3)	2.16,	0.955,	1.135,	1.035	0.885,	0.33,	1.325,	0.77,	0.975,	2.375	0.575	0.985	0.85,	1.39,
	1.89,	-0.545,	0.615		0.685	0.48,	0.98, 0.51,	2.93,	-0.025,			1.21,	0.98	1.125
	2.52,	0.545,				0.13, 0.2	0.2	1.2,	0.42			0.435,		
	1.63,	0.58						0.93,				0.265		

SRC728 (LT4)	0.22, 0.03, 0.41, 0.16	0.005, -1.375, -0.265, 0.12	-0.255, -0.165	-0.345	-0.005, 0.495	-1.04, -0.47, -0.26, -0.41	0.715, -0.38, 0.04, 0.96, 0.28	-0.39, 0.32, 0.31, 0.35, 0.385	0.095, 0.155, 0.41	0.475	0.115	1.335, 0.93, 0.555, -0.395	0.14, 0.31	0.35, 0.775
SRC729 (LT6)	1.66, 0.76, 0.82, -0.02	0.765, -1.505, -0.125, -0.03	1.395, 0.335	0.245	0.335, 0.025	-0.15, -0.35, -0.35, -0.63	1.945, 1.42, 0.02, 0.72, -0.79 0.2,	2.6, 3.18, 0.2, 0.14, 0.025	0.345, 0.035, 0.17	2.795	0.455	0.755, 0.73, 0.365, -0.395	0.35, 0.43	1.67, 0.535
SRC730 (LT7)	1.01, 0.54, 0.69, -0.33	0.835, -1.235, 0.065, 0.3	0.735, 0.085	0.755	-0.465, 0.155	0.42, 0.11, -0.05, -0.05	0.705, -1.16, -0.78, -1.19, -0.38	0.14, 0.14, 0.3, 0.41, 0.015	0.365, 0.265, -0.04	1.115	0.365	0.385, 0.73, 0.485, -0.095	0.29, 0.19	0.95, 0.905
SRC731 (LT9)	1.16, 0.74, 1.11, 0.39	-0.055, -1.035, -0.275, 0.69	0.785, 0.215	-0.345	0.185, 0.255	-0.45, -0.47, -0.21, 0.05	2.645, 0.63, -0.08, -0.94, 0.1	3.47, 2.91, 0.31, 0.35, 0.055	1.005, 0.165, 0.42	2.735	0.655	1.055, 1.14, 0.495, -0.405	-0.09, 0.32	2.9, 0.725
SRC732 (LT10)	1.78, 0.72, 1.22, 0.6	-1.125, -0.155, 0.055, 0.87	1.305, 0.145	0.325	0.265, 0.185	0.42, -0.3, 0.32, 0.03	1.845, 1.13, 0.74, -0.18, 0.3 0.55, 0.5, 0.175	3.42, 3.51, 0.55, 0.5, 0.175	1.125, -0.045, 0.4	3.515	1.135	0.635, 0.9, 0.605, -0.365	0.72, 0.24	2.92, 0.985



SRC733 (LT11)	1.86, 1.42, 1.29	1.21, 0.52	1.53, 0.85	0.7		1.52, 0.135, 0.7	1.005, 0.115, 0.58	1.395, 0.97, 1.35, 0.01	1.185, 2.875, 0.36	1.75, 0.12, 1.12	1.535, 2.935, 1.405	1.525	0.715, 0.82, -0.045, 0.1, 0.21, 0.935	1.03, 0.63	1.515, 1.44, 0.91
SRC734 (LT12)	0.81, 0.69, 0.21	1.98, 0.72	2.99, 2.15	2.17		1.2, 0.225, 0.7	2.225, -0.795, 1.5	1.425, 1.73, -3.18, 0.26	1.225, 2.225, 0.81	1.63, 1.11, 1.14	1.765, 3.015, 1.815	1.195	0.465, 0.69, -0.005, 0.12, -0.16, 1.675	1.18, 0.88	1.755, 1.41, 1.05
SRC735 (LT13)	2.71, 2.24, 1.45	2.72, 1.15	2.48, 1.69	2.24		1.81, 1.175, 1.05	2.705, 0.065, 2.23	2.035, 2.28, -2.17, 1.15	1.585, 1.665, 1.31	2.29, 0.4, 1.83	1.545, 2.135, 2.025	1.635	0.745, 1.03, 0.025, 0.01, 0.23, 1.775	1.33, 0.17	1.875, 1.84, 1.36
SRC736 (LT15)	2.89, 2.79, 2.07	2.22, 2.09	3.99, 2.89	3.51		1.62, -0.165, 1.33	3.615, -0.515, 1.3	1.615, 2.73, 1.27, 1.44	2.205, 2.445, 1.89	2.33, -0.18, 1.89	2.215, 2.005, 2.495	1.775	0.585, 0.74, 0.045, -0.25, 2.375	1.28, 0.52	2.295, 2.24, 2.2

SRC737 (LT16)	1.13, 0.4, 1.78	0.75, 0.99	1.16, 2.65	3.32	1.13, -0.035, 1.09	0.005, -0.405, 1.7	-0.455, 0.71, -0.4, 0.14	0.405, 2.605, 0.67	1.2, 0.09, 1.1	0.605, 2.975, 1.765	0.825	1.115, 1.31, 0.825, -0.2, -0.43 0.295	1.33, 0.41	1.005, 0.84, 0.75
SRC738 (LT17)	2.11, 1.55, 1.23	1.02, 0.01	1.76, 1.09	1.02	1.46, 0.765, 0.91	0.705, 0.095, 1.15	0.365, 1.24, -1.67, 0.88	1.275, 2.855, 1.01	1.95, 0.37, 1.33	0.785, 2.895, 1.615	1.455	0.835, 1.21, 0.425, 0.29, 0.32, 0.535	0.92, 0.81	1.565, 1.49, 1.31
SRC739 (LT18)	0.22, -0.08, 1.72	0.11, 0.86	0.33, 0.53	0.52	0.95, 0.125, 0.72	-0.905, -0.465, 1.17	-0.365, -0.21, -1.8, 0.87	-0.065, 2.455, 0.6	0.25, 0.23, 1.14	-1.215, 3.275, 1.675	1.255	0.135, 0.28, 0.085, -0.26, 0.34, -0.295	0.75, 0.64	0.595, 0.47, 1.12
SRC740 (LT19)	3.51, 3.23	2.33	3.58	---	2.47, 0.565	3.815, -0.495	1.835, 1.35, -2.61	2.295, 2.645	2.38, 0.73	2.535, 2.975, 1.205	---	0.445, 0.36, -0.135, 0.2, -0.31, 2.485	0.44	2.655, 2.67, 0.29

SRC741 (LT21)	1.9, 0.7	1.42	0.67	---	1.09, 0.085	0.075, 0.125	0.075, 1.14, 0.91	0.645, 2.675	0.89, 0.34	-1.905, 3.135	---	0.735, 1, 0.095, 0.35, 0.345	0.26	0.735, 1.03
SRC742 (CT2)	3.81	3.545	3.645	---	1.84	1.95, 3.49	2.1, 1.605	2.01	1.675, 1.605	3.715, 2.55	2.265	0.345	2.4	2.185
SRC743 (CT3)	2.3	0.735	1.125	---	0.56	1.15, 0.54	1.01, 0.825	0.5	1.135, 1.105	3.905, 1.58	0.955	-0.455	1.52	1.645
SRC744 (CT8)	1.97	1.525	1.645	---	0.75	1.29, 1.58	1.3, 0.945	1.1	1.285, 1.345	2.655, 0.91	1.065	0.455	1.55	1.105
SRC745 (CT10)	3.01	1.635	2.535	---	0.85	1.88, 1.49	0.84, 0.435	1.42	2.155, 1.785	3.565, 1.78	1.575	-0.255	1.97	1.645
SRC746 (CT12)	2.46	0.825	1.885	---	0.68	1.74, 0.92	-0.06, -0.135	0.79	0.845, 0.615	3.495, 1.08	1.315	0.355	1.36	1.055
SRC747 (CT14)	3.34	1.585	2.515	---	1.16	2.33, 1.72	1.39, 1.145	1.5	1.265, 1.575	3.875, 1.86	1.895	-0.185	1.75	1.625
SRC748 (CT15)	2.58	0.915	1.305	---	1.17	2.06, 1.41	1.3, 0.845	1.25	1.585, 1.475	3.615, 0.99	1.465	1.165	1.75	1.255
SRC749 (CT16)	2.46	0.675	1.475	---	0.81	1.78, 0.55	1.33, 1.055	1.05	1.095, 1.475	3.115, 0.38	1.255	0.485	1.11	1.295
SRC750 (CT17)	2.64	0.715	1.715	---	0.52	2.4, 0.6	0.77, 0.205	0.85	1.245, 1.375	3.335, 0.3	0.805	1.025	1.11	0.885

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SRC751 (CT1)	1.57	0.735	1.375	---	1.245	0.61, 0.235	0.745, 1.045	1.045	1.285, 1.6, 1.085	0.555	1.245	0.12	1.09	1.17
SRC752 (CT4)	3.39	0.735	2.225	---	1.465	1.77, -0.135	0.435, 0.875	1.275	1.375, 2.23, 1.165	1.105	1.535	-0.9	1.16	1.1
SRC753 (CT5)	2.95	0.665	2.505	---	1.515	2.68, -0.005	1.625, 1.985	1.695	1.975, 2.07, 1.715	2.205	1.975	0.87	1.95	2.03
SRC754 (CT6)	2.81	0.655	2.285	---	0.585	0.74, -0.015	0.035, 0.755	1.085	1.305, 1.73, 1.245	1.115	1.575	1.01	0.96	0.92
SRC755 (CT7)	2.77	0.175	0.945	---	0.015	1.66, 0.065	0.765, 0.105	1.735	1.005, 1.65, 1.025	0.665	0.125	0.93	1.14	0.28
SRC756 (CT9)	2.63	0.435	1.585	---	0.505	-0.4, -0.155	-0.385, 0.955	0.665	0.345, 1, 0.195	1.285	0.975	0.8	0.27	0.72
SRC757 (CT11)	3	0.695	3.335	---	1.355	1.55, 0.125	1.315, 1.525	1.835	2.185, 2.54, 2.145	1.475	2.285	1.34	1.88	2.13
SRC758 (CT18)	2.23	0.465	1.075	---	0.385	0.38, 0.345	0.345, 0.565	0.895	0.645, 1.69, 0.815	0.505	0.845	1.35	0.63	0.77

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PRO201, PRO327 and PRO1265:

PRO201 (UNQ175) (DNA30676), PRO327 (UNQ288)(DNA38113) and PRO1265 (UNQ636)(DNA60764) were also reexamined along with selected tumors from the above initial screen with framework mapping. Figure 32 and Table 3 indicate the chromosomal mapping of the framework markers that were used in the present example. The framework markers are located approximately every 20 megabases and were used to control aneuploidy.

PRO201 (UNQ175)(DNA30676), PRO327 (UNQ288)(DNA38113) and PRO1265 (UNQ636)(DNA60764) were also reexamined with epicenter mapping. The markers indicated in Tables 4A, 4B and 4C are located in close proximity (in the genome) to DNA30676, DNA38113 and DNA60764, respectively, and are used to assess the relative amplification in the immediate vicinity of Chromosome 19 wherein the respective molecule is located. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-35441 is the marker found to be the closest to the location on chromosome 19 where DNA30676 maps, ——— is closest to DNA38113 and SHGC-33698 is closest to DNA60764.

Table 3  
Framework Markers Along Chromosome 19

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name
S12	AFMa107xc9
S50	SHGC-31335
S105	SHGC-34102
S155	SHGC-16175

Table 4A  
Epicenter Markers Along Chromosome 19 used for DNA30676

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
S12	AFMa107xc9	22
S16	SHGC-1261	53
S17	SHGC-2897	7
S18	SHGC-35441	59
S19	SHGC-6150	33
S21	AFM224ye9	21
S23	SHGC-31478	25
S24	SHGC-3921	25

Table 4B  
Epicenter Markers Along Chromosome 19 used for DNA38113

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name	Distance to next Marker (cR <sup>1</sup> )
S42	WI-7289	5
S43	SHGC-32638	28
S44	SHGC-11753 <sup>2</sup>	7
DNA 38113	-	-
S45	SHGC-14810	37
S46	AFM214YF6	15
S48	SHGC-36583	-

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Table 4C

Epicenter Markers Along Chromosome 19 used for DNA60764

Map Position on Chromosome 19	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
DNA34353	—	maps to S158
DNA40620	—	maps to S160
DNA54002	—	maps to S160
S160	SHGC-34723	21
DNA60764	—	—
S161	SHGC-30929	15
S162	SHGC-10328	17
S163	AFMa115wg5	—

The Ct values of the above described framework markers along Chromosome 19 relative to PRO201, PRO327 and PRO1265 are indicated for selected tumors in Table 5A, 5B and 5C, respectively.

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Table 5A

Amplification of framework markers relative to DNA30676 (Ct)

Tumor	Framework Markers				
	S12	DNA30676	S50	S105	S155
LT1	0.16	-0.18	0.06	-0.42	0.11
LT1a	0.05	0.79	-0.27	0.17	0.40
LT2	0.48	-0.09	0.41	0.52	0.13
LT3	0.27	1.04	0.83	0.11	0.50
LT4	0.48	-0.18	0.67	0.20	0.56
LT6	0.72	-0.23	0.74	0.32	0.35

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	LT7	0.82	-0.36	0.85	0.95	0.95
	LT9	0.72	-0.75	0.61	0.19	0.64
	LT10	0.82	0.05	0.98	0.62	0.53
	LT11	0.13	0.64	0.25	0.55	-0.34
5	LT12	0.04	-0.60	0.60	0.21	-0.17
	LT13	-0.06	0.67	0.57	-0.30	-0.05
	LT15	-0.03	1.43	-0.77	0.12	-0.04
	LT16	0.46	1.35	1.37	0.51	0.23
	LT17	0.37	1.51	0.74	0.21	0.22
10	LT18	0.39	1.22	0.57	0.11	0.16
	LT22	0.79	0.13	0.76	-0.05	0.16
	CT2	0.25	2.81	0.29	0.37	-0.02
	CT3	-0.17	2.03	-0.10	0.34	-0.28
	CT8	—	1.39	0.57	0.18	-0.16
15	CT10	0.15	2.21	0.51	-0.01	-0.81
	CT12	0.135	1.93	0.57	0.41	0.20
	CT14	0.40	2.37	0.39	0.45	0.36
	CT15	-0.23	1.27	-0.30	-0.06	0.56
	CT16	0.38	1.76	0.31	0.24	0.04
20	CT17	0.25	1.65	0.71	0.32	0.22

Table 5B  
Amplification of framework markers relative to DNA38113 ( Ct)

Tumor	Framework Markers					
	S12	DNA38113	S50	S105	S155	
25	LT1	0.16	-0.15	0.06	-0.42	0.11
	LT1a	0.05	0.57	-0.27	0.17	0.40
	LT2	0.48	0.57	0.41	0.52	0.13
	LT3	0.27	0.77	0.83	0.11	0.50
	LT4	0.48	0.08	0.67	0.20	0.56
30	LT6	0.72	0.33	0.74	0.32	0.35
	LT7	0.82	0.29	0.85	0.95	0.95
	LT9	0.72	-0.19	0.61	0.19	0.64
	LT10	0.82	1.45	0.98	0.62	0.53
	CT2	0.25	2.94	0.29	0.37	-0.02

5	CT3	-0.17	1.23	-0.10	0.34	-0.28
	CT8	0.13	1.45	0.57	0.18	-0.16
	CT10	0.15	1.72	0.51	-0.01	-0.81
	CT12	0.13	1.60	0.57	0.41	0.20
	CT14	0.40	2.03	0.39	0.45	0.36
	CT15	-0.23	0.68	-0.30	-0.06	0.56
	CT16	0.38	1.07	0.31	0.24	0.04
	CT17	0.25	0.50	0.71	0.32	0.09

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Table 5C

Amplification of framework markers relative to DNA60764 ( Ct)

Tumor	Framework Markers					
	S12	DNA60764	S50	S105	S155	
15	LT1	0.16	0.06	-0.42	0.11	-1.56
	LT1a	0.05	-0.27	0.17	0.40	0.00
	LT2	0.48	0.41	0.52	0.13	-0.36
	LT3	0.27	0.83	0.11	0.50	1.04
	LT4	0.48	0.67	0.20	0.56	-0.35
20	LT6	0.72	0.74	0.32	0.35	0.24
	LT7	0.82	0.85	0.95	0.95	0.75
	LT9	0.72	0.61	0.19	0.64	-0.35
	LT10	0.82	0.98	0.62	0.53	0.32
	LT11	0.13	0.25	0.55	-0.34	0.70
25	LT12	0.04	0.60	0.21	-0.17	2.17
	LT13	-0.06	0.57	-0.30	-0.05	2.24
	LT15	-0.03	-0.77	0.12	-0.04	3.51
	LT16	0.46	1.37	0.51	0.23	3.32
	LT17	0.37	0.74	0.21	0.22	1.02
	LT18	0.39	0.57	0.11	0.16	0.52
	LT22	0.79	0.76	-0.05	0.16	0.59

30 Table 6. 6.1 and 6.2 indicate the Ct values for results of epicenter mapping relative to DNA30676, DNA38113 and DNA60764 respectively, indicating the relative amplification in the region more immediate to the actual location of DNA30676, DNA38113 and DNA60764 along chromosome 19.

Table 6

Amplification of epicenter markers relative to DNA30676 ( C1)

	Tumor	S12	S16	S17	S18	DNA30676	S19	S21	S23	S24
5	LT1	---	0.22	-0.16	0.02	-0.29	0.00	0.40	-0.02	0.14
	LT1a	---	0.11	-0.52	0.32	0.58	0.00	-0.55	0.04	-0.15
	LT2	---	-0.07	-0.07	0.34	-0.04	0.00	0.07	0.13	0.12
	LT3	---	0.01	-0.46	0.47	1.87	0.00	0.16	0.24	0.02
	LT4	---	-0.36	-0.96	0.93	-1.18	0.00	-0.54	-0.07	-0.23
10	LT6	---	-0.35	-0.70	-0.04	0.28	0.00	-0.24	-0.12	-0.01
	LT7	---	-0.32	-0.34	-0.27	0.29	0.00	-0.74	-0.07	0.05
	LT9	---	-0.42	-0.66	-0.36	0.07	0.00	-1.42	-0.26	-0.70
	LT10	---	-0.26	-0.14	-0.07	0.55	0.00	-0.32	-0.04	-0.08
	LT11	---	-0.22	-0.77	0.05	0.68	0.00	-0.85	-0.13	0.09
15	LT12	---	-0.94	-1.52	-1.26	0.13	0.00	-0.08	-0.09	0.24
	LT13	---	0.24	0.02	0.35	1.44	0.00	-0.08	0.50	0.49
	LT15	---	-0.09	-0.64	0.26	1.99	0.00	0.03	0.09	-0.06
	LT16	---	0.06	-0.16	0.20	1.72	0.00	0.75	0.54	0.64
	LT17	---	-0.91	-1.71	-0.78	-0.15	0.00	-2.89	-0.82	-0.42
20	LT18	---	0.30	-0.20	0.71	1.09	0.00	-0.29	0.34	0.80
	LT22	---	0.37	-0.82	0.47	0.07	0.00	0.46	0.38	0.65
	CT1	0.46, 0.18	0.02, 0.19	0.35, 0.32	0.59, 0.57	3.51, 1.61	---	-0.15, 0.75	0.53, 0.56	0.05, 0.14
	CT3	-0.02	-0.24	0.05	0.13	2.19	---	-0.31	0.13	-0.34
	CT4	0.29	0.20	0.42	0.64	3.22	---	0.47	0.27	0.33
25	CT5	-0.15	-0.16	0.12	-0.21	2.83	---	0.09	-0.08	-0.17
	CT6	0.13	0.17	0.87	0.26	2.93	---	0.44	0.04	0.39
	CT7	0.13	-0.03	0.78	-0.04	2.43	---	-0.68	-0.26	0.20
	CT8	0.45	-0.03	0.58	0.22	1.95	---	0.25	0.57	0.07
	CT9	0.50	0.41	0.98	0.64	2.72	---	0.24	0.06	0.66
30	CT10	0.11	-0.40	0.32	0.13	3.12	---	-0.16	0.28	-0.16
	CT11	0.18	0.01	0.45	0.82	3.26	---	0.34	0.00	0.27
	CT12	0.53	0.08	0.72	0.40	2.77	---	0.36	0.67	0.09
	CT14	0.57	-0.13	0.87	0.63	2.88	---	0.59	0.74	0.09
	CT15	-0.09	-0.57	0.05	0.11	2.60	---	-0.07	0.20	-0.34
	CT16	0.57	-0.21	0.80	0.36	2.61	---	0.38	0.49	0.16

CT17	0.25	-0.26	0.38	0.29	2.24	---	-0.05	0.67	0.05
CT18	0.38	0.18	0.53	0.49	2.48	---	0.41	-0.29	0.12

Table 6.1

Amplification of epicenter markers relative to DNA38113 ( Ct)

5	Tumor	S41	S42	S43	S44	DNA38113	S45	S46	S48
	LT1	-1.03	-0.25	-0.18	-0.11	-0.31	0.13	0.26	0.29
	LT1a	0.14	-0.30	-0.11	-0.01	0.21	-0.44	0.45	-0.30
	LT2	0.03	0.06	0.06	0.12	0.14	0.16	0.11	0.65
10	LT3	-1.08	-0.08	-0.01	0.11	0.43	-0.37	0.33	0.56
	LT4	0.66	-0.14	-0.48	-0.79	-0.28	-0.31	0.04	0.09
	LT6	-0.88	-0.08	-0.12	-1.00	0.20	-0.43	0.48	0.63
	LT7	0.65	-0.19	-0.19	-0.04	0.04	-0.42	0.43	0.57
	LT9	0.66	-0.26	-0.01	-0.14	-0.06	-0.31	-16.48	0.16
15	LT10	1.16	-0.30	-0.11	-0.31	0.13	-0.33	0.34	0.50
	LT11	0.46	0.01	-0.04	-0.86	0.67	0.23	0.24	-0.57
	LT12	1.39	-0.01	-0.22	-1.33	1.57	-0.25	0.26	0.07
	LT13	1.62	-0.03	0.00	-0.08	1.22	-0.08	0.48	0.14
	LT15	1.09	0.20	0.47	0.62	2.47	0.38	0.01	0.44
20	LT16	1.51	0.04	-0.04	0.29	2.23	0.51	0.50	0.90
	LT17	2.12	0.23	0.11	0.20	1.02	0.45	0.46	-0.41
	LT18	1.80	-0.11	0.07	-0.70	0.9	0.10	0.00	-0.02
	LT22	-0.12	0.06	0.41	-0.11	-0.06	0.34	0.03	0.52
	CT1	-0.09	0.33	0.11	0.22	1.38	0.09	-0.25	-0.10
25	CT2	1.76	0.04	0.30	0.65	2.94	0.18	-0.04	0.01
	CT3	1.10	-0.31	-0.24	0.16	1.23	-0.64	0.78	-0.17
	CT4	1.63	0.22	0.32	-0.72	2.23	-0.04	0.44	0.72
	CT5	2.22	0.02	0.21	0.10	2.51	0.02	0.18	0.24
	CT6	0.48	0.20	0.22	-0.63	2.29	0.03	0.14	0.97
30	CT7	0.93	0.20	0.32	0.14	0.95	-0.01	0.20	0.54
	CT8	1.15	-0.50	-0.14	0.15	1.45	-0.31	0.54	0.07
	CT9	0.82	0.38	0.64	-0.71	1.59	1.04	0.26	0.93
	CT10	1.57	-0.41	-0.03	-0.14	1.72	-0.27	0.04	0.10
	CT11	1.49	-0.05	0.07	0.01	3.34	0.54	0.28	0.88

CT12	0.89	-0.09	-0.01	-0.62	1.6	-0.07	1.16	0.92
CT14	2.16	0.32	0.37	0.47	2.03	-0.07	1.21	0.44
CT15	0.64	-0.52	-0.21	-0.12	0.68	-0.61	1.01	0.32
CT16	1.75	-0.31	0.28	0.47	1.07	0.04	1.01	-0.29
5 CT17	0.77	-0.18	0.13	-0.04	0.5	-0.27	0.93	0.31
CT18	0.91	0.05	0.14	0.60	1.08	0.22	-0.59	0.61

Table 6.2 indicates the Ct values for the results of epicenter mapping relative to DNA60764, indicating relative amplification in the region more immediate to the actual location of DNA60764 along chromosome 19.

- 10 DNA34353, DNA40620 and DNA54002 are other independently identified molecules which have been observed to map to the same region of chromosome 19 as DNA60764.

Table 6.2  
Amplification of epicenter markers relative to DNA60764 (Ct)

15	Tumor	DNA34353	DNA40620	DNA54002	S160	DNA60764	S161	S162	S163
	LT1	-0.22	-0.27	-2.62	0.03	-1.26	0.00	-0.33	-0.29
	LT1a	0.49	-0.08	—	0.13	-0.02	0.00	-0.26	-0.64
	LT2	0.47	-0.22	-0.22	0.04	-0.09	0.00	-0.20	-0.23
	LT3	0.81	-0.19	0.07	0.07	0.32	0.00	0.27	0.15
20	LT4	0.64	-0.16	0.04	0.37	-0.73	0.00	-0.12	-0.64
	LT6	0.44	-0.36	-0.36	-0.20	-0.22	0.00	-0.12	-0.64
	LT7	0.54	0.18	0.23	0.22	-0.47	0.00	0.16	-0.03
	LT9	0.40	-0.26	0.13	-0.10	-0.27	0.00	-0.71	-0.24
	LT10	0.89	0.10	-0.81	0.25	0.57	0.00	0.11	0.01
25	LT11	0.17	-0.43	0.22	-0.20	0.47	0.00	-0.06	0.30
	LT12	0.91	-0.04	0.64	0.27	2.35	0.00	0.51	0.58
	LT13	0.81	0.05	0.74	0.10	2.37	0.00	0.21	0.60
	LT15	1.03	-0.06	0.54	0.49	3.88	0.00	0.21	0.64
	LT16	1.22	0.40	0.86	0.63	3.32	0.00	0.50	0.81
30	LT17	1.02	0.13	0.35	-0.39	0.88	0.00	0.23	0.73
	LT18	0.72	-0.32	-0.20	-0.30	0.18	0.00	-0.12	-0.04

LT22	-0.18	-0.54	0.56	-0.14	-0.75	0.00	0.02	-0.34
CT1	0.46	0.19	0.43	0.31	1.74	—	0.16	0.38
CT4	0.71	0.15	0.38	0.19	1.86	—	0.25	0.61
CT5	1.20	0.04	0.41	0.48	3.28	—	0.29	0.73
CT6	0.86	-0.04	0.45	0.36	0.93	—	-0.07	0.48
CT7	0.81	-0.07	0.02	-0.21	1.29	—	-0.16	0.38
CT9	1.00	0.43	0.18	0.50	-0.25	—	0.34	0.72
CT11	1.11	0.07	0.68	0.30	2.32	—	0.05	0.31
CT18	0.83	-0.10	0.11	0.21	0.39	—	-0.04	0.04

PRO292:

PRO292 (UNQ266) (DNA35617) was also examined with framework mapping. Figure 33 and Table 7 indicate the chromosomal mapping of the framework markers that were used in this analysis. The framework markers are located approximately every 20 megabases and were used to control aneuploidy

Table 7  
Framework Marker used on Chromosome 11 for DNA35617

Map Position on Chromosome 11	Stanford Human Genome Center Marker Name
K7	SHGC-14668
K62	SHGC-31021
K113	SHGC-6028
K162	SHGC-11920
K226	SHGC-6023
K282	SHGC-9000
K326	SHGC-12291
K365	SHGC-10796
K412	SHGC-6005

The  $\Delta C_t$  values of the above described framework markers along Chromosome 11 relative for PRO292 are indicated for selected tumors in Table 8.

Table 8  
Amplification of Framework Markers relative to DNA35617

Tumor	DNA35617	K7	K62	K113	K162	K226	K282
LT1	-1.56	-0.15	-2.31	-0.42	-0.44	-2.14	-0.57

	LT1a	-0.61	-1.11	-0.50	-1.71	-0.76	-0.09	-0.07
	LT2	-0.23	0.00	-0.57	0.39	-0.20	0.12	0.09
	LT3	0.39	-0.02	-0.38	0.08	-0.17	0.24	0.06
	LT4	-0.18	0.21	-1.54	0.82	-0.35	0.66	0.19
5	LT6	-0.34	0.10	-0.64	-2.51	-0.24	0.29	-0.01
	LT7	-0.45	-0.08	-0.44	0.56	0.08	-0.24	-0.65
	LT9	-0.36	-0.36	-1.63	0.36	-0.15	0.58	-0.11
	LT10	-0.23	-0.07	-0.82	0.42	-0.87	0.24	-0.48
	LT11	0.76	-0.24	0.71	0.00	-0.57	0.30	-0.09
10	LT12	1.61	-0.61	2.36	0.00	-0.55	-0.47	-0.22
	LT13	1.95	-0.66	2.53	0.00	-0.64	-0.83	-0.13
	LT15	2.86	-0.19	3.21	0.00	-0.68	0.01	-1.19
	LT16	1.68	-0.65	3.02	0.00	-1.01	0.10	-0.29
	LT17	0.72	-0.21	-0.48	0.00	-0.32	-0.04	-0.20
15	LT18	0.34	0.13	-1.15	0.00	-0.25	-0.41	-0.13
	LT22	-0.57	0.37	-0.46	0.00	0.15	0.26	0.57
	CT2	3.75	0.40	4.19	0.28	0.27	0.47	0.26
	CT3	0.63	0.16	0.75	0.10	0.04	0.28	0.19
	CT8	1.76	0.18	1.21	0.79	-0.05	0.41	0.19
20	CT10	1.68	-0.32	1.98	-0.12	-0.32	0.23	-0.08
	CT12	0.77	0.48	1.08	0.55	-0.36	0.35	0.15
	CT14	1.75	-0.09	2.02	0.37	0.56	0.94	0.37
	CT15	0.90	-0.11	0.56	0.37	0.02	0.48	0.05
	CT16	0.80	0.12	0.76	0.02	-0.06	0.20	0.03
25	CT17	0.82	0.48	0.57	-0.07	-0.02	0.00	-0.27

PRO343 and PRO882:

PRO343 (UNQ302) (DNA43318) and PRO882 (UNQ448) (DNA58125) were also examined with both framework and epicenter mapping. Figure 34 and Table 9 indicate the chromosomal mapping of the framework markers that were used in this analysis. The framework markers are located approximately every 20 megabases and were used to control aneuploidy. Tables 9.1 and 9.2 indicate the epicenter markers used for the mapping of DNA43318 and DNA58125. The markers shown in Tables 9.1 and 9.2 are located in close proximity (in the genome) to DNAs DNA43318 and DNA58125, respectively, and are used to assess the relative amplification in the immediate vicinity of Chromosome 16 wherein the respective molecules map. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit roughly

equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The markers AFMa061yb5 and SHGC-36123 are located the closest to the location on chromosome 16 where DNA43318 and DNA58125, respectively, map.

Table 9

5

**Framework Marker used on Chromosome 16 for DNA43318 and DNA58125**

Map Position on Chromosome 16	Stanford Human Genome Center Marker Name
P7	SHGC-2835
P55	SHGC-9643
P99	GATA7B02
P154	SHGC-33727
P208	SHGC-13574

### Table 9.1

15

**Epicenter markers Along Chromosome 16 used for DNA43318**

Map Position on Chromosome 16	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
P106	AFM210yg3	15
P107	CHLC.GATA81B12	17
P108	WI-1256	17
DNA43318	—	—
P109	AFMa061yb5	20
P110	CHLC.GGAA23C09	31
P111	AFMa183wd9	12
P112	WI-7078	—

### Table 9.2

25

**Epicenter markers along Chromosome 16 used for DNA58125**

Map Position on Chromosome 16	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
P89	SHGC-11302	27
P90	EST00087	8
P92	SHGC-2726	23
DNA58125	—	—
P93	SHGC-36123	42
P94	SHGC-35326	23
P95	IB391	—



The  $\Delta C_t$  values of the framework markers of Table 9 along Chromosome 16 relative for PRO343 and PRO882 are indicated for selected tumors in Table 10.

Table 10  
Amplification of Framework Markers relative to DNA43318 and DNA58125

	Tumor	P7	P55	DNA58125	P99	DNA43318	P154	P208
5	LT1	-3.62	-0.07	0.18	0.03	-1.04	-0.22	-0.06
	LT1a	-1.90	-0.13	0.75	0.10	-0.20	0.45	0.28
	LT2	-0.41	-0.05	0.36	0.07	-0.44	-0.07	0.41
	LT3	0.18	-0.37	1.02	-0.17	0.21	-0.18	0.19
10	LT4	-3.58	-0.25	0.65	-0.13	-0.38	-0.05	0.04
	LT6	-0.57	-0.26	0.34	0.05	-0.33	-0.23	0.09
	LT7	-1.60	-0.46	0.43	1.14	-0.37	0.25	-0.54
	LT9	-0.77	-0.14	0.36	0.33	-0.39	-0.18	0.43
	LT10	-2.60	-0.28	0.50	0.20	-0.12	-0.02	0.39
15	LT11	-0.64	-0.15	0.86	-0.02	0.35	-0.08	-0.55
	LT12	-1.19	-0.11	1.00	-0.50	1.16	-0.74	-0.97
	LT13	-0.31	-0.27	1.33	0.02	1.72	-0.38	-0.40
	LT15	-0.90	-1.90	1.83	-0.07	2.73	-0.18	-0.39
	LT16	-1.29	-0.92	0.97	-0.68	1.46	-0.43	-0.90
20	LT17	-0.13	-0.15	1.03	0.02	0.42	-0.15	-0.52
	LT18	-1.24	-0.43	1.08	-0.04	1.06	-0.13	-0.45
	LT22	-1.86	-0.29	0.05	-0.09	-0.66	-0.12	-0.26
	CT1	-0.73	0.35	1.08	-0.09	0.24	0.05	-0.03
	CT2	2.72	0.93	2.27	0.72	3.54	0.48	-0.13
25	CT3	0.01	0.07	1.34	0.53	0.42	-0.27	-0.52
	CT4	-0.99	-0.07	1.13	-0.61	1.17	-0.43	-0.09
	CT5	0.09	0.34	2.17	-0.04	2.63	-0.19	-0.01
	CT6	-1.36	-0.29	1.41	-0.03	0.46	-0.16	0.27
	CT7	-1.36	0.09	0.24	-0.18	-0.81	-0.17	-0.13
30	CT8	-1.01	1.05	1.23	0.69	1.44	0.60	0.04
	CT10	0.95	0.84	1.74	0.75	1.36	-0.17	-0.57
	CT12	-0.73	0.49	1.13	0.71	0.87	0.60	-0.88
	CT14	-0.16	1.49	1.74	0.83	1.68	0.33	-0.38
	CT15	-1.23	0.72	1.30	0.60	1.07	-0.29	-0.70
35	CT16	0.05	1.07	0.93	0.59	1.01	-0.13	-0.66

CT17	0.27	1.06	0.91	0.83	0.67	-0.15	-0.77
CT18	0.32	0.81	1.04	0.74	0.79	0.55	0.36

Table 10.1 and 10.2 indicate the  $\Delta C_t$  values from epicenter mapping relative to DNA43318 and DNA58125, respectively, indicating the relative amplification in the region more immediate to the actual location of the  
5 respective molecules along chromosome 16.

Table 10.1  
Amplification of epicenter markers relative to DNA43318 ( $\Delta C_t$ )

	Tumor	Epicenter marker							
		P106	P107	P108	DNA4331 8	P109	P110	P111	P112
5	LT1	-0.30	0.56	-0.43	-1.50	-0.30	-1.52	-0.04	-0.09
	LT1a	-0.80	0.79	0.48	-0.47	0.45	-1.52	-0.04	-0.09
	LT2	-0.17	0.43	-0.17	-0.52	0.14	-1.52	1.05	0.47
	LT3	-0.46	-0.53	-0.49	0.18	-0.02	-1.52	-0.03	-0.24
	LT4	-0.06	0.56	-0.61	-0.77	-0.79	-1.52	0.38	-0.39
10	LT6	-0.19	1.24	-0.46	-0.38	-0.55	-1.52	0.38	-0.39
	LT7	0.56	1.52	0.30	-0.43	1.02	-1.52	1.58	0.94
	LT9	-0.47	-0.16	-0.13	-0.41	0.64	-0.12	0.32	-0.22
	LT10	-0.24	-1.00	-0.35	-0.22	-1.54	-1.52	0.22	-0.16
	LT11	0.18	0.79	-0.38	0.25	0.22	0.00	-0.01	0.00
15	LT12	-0.61	0.47	-0.32	1.32	-0.09	0.00	-1.01	-0.37
	LT13	0.28	1.41	0.03	1.94	-0.44	0.11	0.11	0.39
	LT15	-0.23	-1.29	0.10	3.07	-0.80	0.00	-0.42	-0.01
	LT16	-0.44	-0.18	-0.53	1.83	-0.96	0.00	-0.15	-0.18
	LT17	0.61	0.39	-0.12	0.45	-0.29	0.00	-0.13	0.24
20	LT18	0.26	0.77	-0.16	0.11	-1.07	0.00	-0.12	0.15
	LT22	0.31	0.91	0.22	-0.52	0.11	0.00	0.24	0.21
	CT1	0.17	1.09	-0.13	0.10	0.98	0.00	-0.13	-0.04
	CT4	-0.63	0.69	0.07	1.02	0.61	0.00	-0.14	-0.06
	CT5	0.03	0.78	-0.17	2.40	0.60	0.00	-0.28	-0.11
25	CT6	-0.21	-1.03	-0.38	3.78	-0.40	0.00	-0.84	-0.22
	CT7	0.03	0.51	-0.08	0.64	0.16	0.00	0.00	-0.11
	CT9	0.26	0.26	-0.55	-0.91	0.46	0.00	0.11	-0.09
	CT11	0.68	1.20	-0.08	1.51	0.68	0.00	0.09	0.05
	CT18	-0.13	1.13	0.09	0.46	0.96	0.00	-0.18	0.13
30	CT2	-0.47	0.19	-0.26	2.48	-0.39	0.00	-0.39	0.41
	CT3	-0.58	1.33	0.03	-1.74	0.99	0.00	0.47	0.03
	CT8	0.39	1.69	0.34	-1.04	1.12	0.00	0.11	0.34
	CT10	0.03	0.29	0.54	-0.98	1.60	0.00	0.33	0.48

CT12	0.25	1.17	0.59	-1.61	0.64	0.00	0.54	0.52
CT14	0.41	1.71	0.38	-0.70	1.75	0.00	0.86	0.62
CT16	0.07	1.15	0.20	-1.41	0.71	0.00	0.14	0.43
CT17	-0.13	0.91	0.29	-1.74	0.60	0.00	0.63	0.37

5

Table 10.2

Amplification of epicenter markers relative to DNA58125 ( $\Delta Ct$ ).

Tumor	Epicenter marker					
	P89	P92	DNA58125	P93	P94	P95
LT1	-0.11	-0.10	-0.02	-0.52	-0.01	-0.13
LT1a	-0.03	0.06	0.65	0.19	-0.33	-0.25
10 LT2	0.02	0.17	0.38	-0.32	0.11	-0.13
LT3	-0.15	0.05	0.77	0.10	0.13	0.04
LT4	0.08	0.02	0.36	-0.72	0.15	-0.43
LT6	-0.82	-0.40	0.07	-1.18	0.09	0.23
LT7	0.09	-0.04	0.41	0.03	0.29	0.32
15 LT9	-0.09	0.12	0.40	0.04	0.18	0.09
LT10	-1.65	-0.79	-0.43	-0.78	0.00	-0.93
LT11	0.15	0.17	0.91	0.10	0.23	0.31
LT12	-1.03	-0.07	1.02	-0.30	0.29	0.27
LT13	0.42	0.44	1.52	-0.12	0.23	0.27
20 LT15	0.48	0.35	2.04	0.37	0.00	0.22
LT16	-0.09	-0.47	1.09	-0.62	0.32	0.54
LT17	0.81	0.46	1.32	0.72	0.46	0.45
LT18	-0.10	-0.35	0.56	-0.56	0.33	-0.53
LT22	0.75	0.67	0.22	0.14	0.13	-0.16
25 CT1	0.40	0.22	2.29	0.33	0.21	0.68
CT4	-0.20	-0.21	1.49	0.81	0.13	-0.07
CT5	0.25	0.17	0.71	-0.30	0.14	-0.12
CT6	0.38	0.39	1.83	0.31	0.21	0.01
CT7	0.37	0.19	1.20	0.44	0.27	-0.12
30 CT9	0.53	0.47	1.67	0.52	0.20	0.20
CT11	0.10	0.09	1.02	0.18	0.05	-0.08

CT18	0.02	0.12	0.78	0.21	0.05	-0.07
CT2	0.17	0.18	1.07	0.41	0.17	0.05
CT3	-0.73	-0.50	0.66	-1.04	0.21	-0.61
CT8	0.54	0.59	2.27	0.76	0.46	0.52
CT10	0.46	0.29	1.50	0.32	0.46	0.12
CT12	0.09	-0.15	0.81	0.05	0.57	0.01
CT14	0.37	0.22	0.47	-0.84	0.50	0.43
CT16	0.50	0.14	2.24	0.15	0.64	0.08
CT17	0.15	0.26	0.82	-0.42	0.07	-0.02

10 PRO1017:

PRO1017 (UNQ500) (DNA56112) was also examined with framework mapping. Figure 35 and Table 11 indicate the chromosomal mapping of the framework markers that were used in this analysis. The framework markers are located approximately every 20 megabases and were used to control aneuploidy.

PRO1017 (UNQ500)(DNA56112) was also examined with epicenter mapping. Table 11.1 indicates the epicenter markers which are located in close proximity to DNA56112 which were employed to assess the relative amplification in the immediate vicinity of chromosome 7 wherein DNA56112 is located. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-22698 is the marker found to be the closest to the location on chromosome 7 where DNA56112 maps.

Table 11  
Framework Marker used on Chromosome 7 for DNA 56112

Map Position on Chromosome 7	Stanford Human Genome Center Marker Name
G11	xAFM210xc7
G54	Z38558
G113	WI-7675
G164	SHGC-33722
G205	WI-1004
G254	AFM036XG5
G302	WI-1841
G358	SHGC-35064
G419	Cda16c10

Table 11.1  
Epicenter Markers along Chromosome 7 used for DNA56112

	Map position on chromosome 7	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
	G5	SHGC-32510	48
	G6	sWSS918	19
	G7	AFMc027xb5	18
5	G8	SHGC-33698	23
	DNA56112	—	—
	G9	EST00439	63
	G12	SHGC-30897	—

10 Table 11.2 indicated the DCt values for the results of epicenter mapping relative to DNA56112, indicating the relative amplification in the region more immediate to the actual location of DNA56112 along chromosome 7.

Table 11.2  
Amplification of epicenter markers relative to DNA56112 ( $\Delta C_t$ )

	tumor	Epicenter markers							
		G5	G6	G7	G8	DNA56112	G9	G11	G12
15	LT1	0.22	0.33	0.37	0.21	0.47	-2.66	0.18	0.34
	LT1a	-0.03	0.27	0.28	0.28	-1.38	-1.64	0.24	0.23
	LT2	-0.08	0.27	0.28	0.28	-1.38	-1.64	0.24	0.23
	LT3	-0.32	0.02	0.34	0.23	0.23	-0.44	0.01	0.32
	LT4	-0.04	-0.08	0.68	0.04	0.65	-0.19	0.10	0.32
20	LT6	-0.42	-0.90	0.14	-0.19	-1.09	-1.17	-0.10	-0.43
	LT7	0.08	0.03	0.21	0.27	0.59	-1.41	0.16	0.28
	LT9	-0.17	-0.09	0.22	0.05	0.04	-1.23	0.13	-0.02
	LT10	-0.09	0.09	0.22	0.22	0.59	-2.19	0.14	0.09
	LT11	0.23	0.32	0.03	0.10	0.64	-0.77	0.31	-0.04
25	LT12	-0.13	0.06	0.02	0.24	1.47	-0.78	0.44	0.16
	LT13	-0.05	0.06	-0.09	0.03	1.51	-0.07	0.23	-0.04
	LT15	-0.18	-0.04	0.07	-0.11	2.27	-0.03	0.20	-0.16
	LT16	0.20	0.06	0.31	0.05	1.62	-0.70	0.54	0.32
	LT17	0.09	-0.04	0.37	-0.04	0.24	-0.98	0.08	0.11
30	LT18	0.00	0.02	-0.04	-0.27	1.03	-1.61	0.29	-0.16
	LT22	0.41	0.48	0.11	0.28	0.28	-2.41	0.53	0.25
	CT1	0.06	0.23	-0.18	0.28	0.23	-1.14	—	-0.02

5	CT4	0.00	0.18	0.16	0.40	0.90	-0.81	—	0.36
	CT5	0.01	0.11	-0.12	0.18	1.29	-0.63	—	-0.48
	CT6	-0.09	-0.07	-0.37	0.03	0.31	-2.83	—	0.13
	CT7	0.01	0.14	0.08	0.22	0.70	-0.08	—	0.23
	CT8	0.17	0.29	0.26	-0.17	1.46	-1.22	0.16	0.43
10	CT9	0.79	0.91	0.38	0.67	0.28	-2.21	—	0.63
	CT10	-0.20	-0.05	0.33	-0.02	2.28	0.60	-0.11	0.43
	CT11	0.02	0.31	-0.02	-0.01	0.96	-1.09	—	0.02
	CT12	0.06	0.27	0.06	0.18	1.84	-0.59	0.38	0.44
	CT14	0.38	0.16	0.50	0.14	2.56	-0.96	0.20	0.56
	CT16	0.05	0.51	0.59	0.22	1.22	0.11	0.18	0.31
	CT17	-0.02	0.42	0.28	0.10	1.52	-0.32	0.22	0.21
	CT18	-0.09	0.34	0.39	0.09	0.46	2.36	—	0.09

The  $\Delta C_t$  values of the above described framework markers along Chromosome 7 relative DNA56112 is

indicated for selected tumors in Table 12.

Table 12  
Amplification of Framework Markers relative to DNA56112

Tumor	DNA56112	G11	G54	G113	G164	G205	G254	G358
LT1	0.60	0.20	0.19	0.00	-0.10	-0.29	-0.26	0.72
LT1a	-1.37	0.20	0.00	0.00	-0.03	-0.67	-1.01	0.42
LT2	0.96	0.27	0.01	0.00	-0.54	-1.28	-1.44	-2.86
LT3	1.21	-0.42	-0.25	0.00	-1.17	-1.17	-1.12	-0.62
LT4	1.59	0.83	0.98	0.00	0.20	-0.78	-0.41	-0.90
LT6	-0.35	-0.21	-0.58	0.00	-2.80	-1.16	-0.53	0.33
LT7	1.40	0.52	0.10	0.00	-0.12	-0.44	-0.36	1.45
LT9	1.07	-0.43	-0.17	0.00	-0.68	-0.10	0.33	1.71
LT10	1.98	0.24	0.01	0.00	0.41	0.22	0.32	2.47
LT11	1.15	-0.56	-0.23	0.00	0.38	-0.18	0.28	-0.01
LT12	2.31	-0.09	0.04	0.00	-0.26	-0.32	-0.11	-0.55
LT13	1.83	-0.55	0.14	0.00	0.27	0.04	0.01	-0.09
LT15	2.79	-0.20	-0.64	0.00	-0.15	-0.59	-0.29	-0.63
LT16	2.22	-0.19	0.18	0.00	-0.51	-0.30	0.06	-0.17
LT17	0.92	0.26	-0.02	0.00	-0.53	-0.66	-0.63	-0.45

LT18	1.06	-1.50	-0.05	0.00	-1.64	-0.86	-1.04	-0.61
LT22	1.18	0.76	0.48	0.00	0.48	0.02	-0.08	0.12

PRO715 and PRO853:

PRO715 (UNQ383) (DNA52722) and PRO853 (UNQ419) (DNA48227) were also reexamined with both framework and epicenter mapping. Figures 36A and 36B and Table 13 indicate the chromosomal localizations of the framework markers that were used for the procedure. The framework markers are located approximately every 20 bases and were used to control aneuploidy. Tables 14A and 14B indicate the epicenter mapping markers that were used in the procedure. The epicenter markers were located in close proximity to DNA52722 and DNA48226, respectively, and are used to determine the relative DNA amplification in the immediate vicinity of DNA52722 and DNA48226. The distance between individual markers is measured in centirays, which is a radiation breakage unit approximately equal to a 1% chance of a breakage between two markers. One cR is very roughly equivalent to about 20 kilobases. In both figures 14A and 14B, "BAC" means bacterial artificial chromosome. The ends of a BAC clone which contained the gene of interest were sequenced. TaqMan primers and probes were made from this sequence, which are indicated in the respective tables. BAC clones are typically 100 to 150 Kb, so these primers and probes can be used as nearby markers to probe DNA from tumors. In figure 14A, the marker SHGC-31370 is the marker found to be the closest to the location on chromosome 17 where DNA52722 maps. In figure 14B, the marker SHGC-37126 is the marker found to be the closest to the location on chromosome 17 where DNA48227 maps.

Table 13

Framework Markers Used Along Chromosome 17 for DNA52722 and DNA48227

Map Position on Chromosome 17	Stanford Human Genome Center Marker Name
Q4	SHGC-31242
Q52	SHGC-35988
Q110	AFM200zf4
Q169	SHGC-32689
Q206	SHGC-11717
Q232	SHGC-32338

Table 14A

Epicenter Markers Used on Chromosome 17 in Vicinity of DNA52722

Map Position on Chromosome 17	Stanford Human Genome Marker Name	Distance to next Marker (cR)
Q33	SHGC-35547	18 cR to Q34
120F17FOR1	Marker from forward end of BAC sequence	



120F17FOR2	Marker from forward end of BAC sequence	
DNA52722	-----	
120F17REV1	Marker from reverse end of BAC sequence	
120F17REV2	Marker from reverse end of BAC sequence	
5 Q34	SHGC-31370	

Table 15A

Epicenter Markers Used on Chromosome 17 in Vicinity of DNA48227

Map Position on Chromosome 17	Stanford Human Genome Marker Name	Distance to next Marker (cR)
10 Q74	AFM238yb10	3
Q73	SHGC-33634	3
203J20FOR1	Marker from forward end of BAC sequence	
203J20FOR2	Marker from forward end of BAC sequence	
Q72	SHGC-37126	
15 DNA48227	---	
203J20REV1	Marker from reverse end of BAC sequence	
203J20REV2	Marker form reverse end of BAC sequence	

Table 16 indicates the  $\Delta C_t$  values of the above described framework markers along chromosome 17 relative to DNA52722 and DNA48227 for selected tumors. While not shown, the similar  $\Delta C_t$  values for the framework markers in the analysis of DNA48227 were reported.

Table 16

Amplification of Framework Markers Relative to DNA52722

Tumor	Framework Marker						
	Q4	Q52	DNA5272	Q110	Q169	Q206	Q232
			2				

5	LT1	0.02	-0.50	-0.04	0.05	-0.32	-0.21	-0.34
	LT1a	-0.01	-0.34	0.64	0.23	-0.20	-0.25	-0.15
	LT2	0.25	0.15	0.19	0.05	-0.16	-0.14	-0.09
	LT3	-0.08	-0.20	0.54	0.56	-0.06	0.32	0.05
	LT4	-0.32	-0.45	0.31	0.19	-0.06	-0.12	0.04
10	LT6	-0.21	-0.38	0.31	0.13	-0.08	-0.30	0.01
	LT7	-0.66	-1.02	0.02	0.62	-0.20	0.06	0.16
	LT9	-0.03	-0.29	0.46	1.20	-1.75	-0.22	-0.13
	LT10	-0.16	-0.09	0.58	0.11	0.01	-0.33	-0.45
	LT11	-0.14	0.29	1.03	0.04	0.30	0.52	0.17
15	LT12	-0.25	-0.68	0.72	0.65	0.86	0.97	0.58
	LT13	0.20	0.00	1.37	-0.15	-0.04	0.25	-0.01
	LT15	0.11	-0.39	1.75	0.00	-0.02	0.43	-0.19
	LT16	-0.07	-0.56	1.11	0.22	0.19	0.68	-0.55
	LT17	0.41	-0.09	1.14	0.27	0.22	0.73	0.07
20	LT18	0.14	-0.22	1.04	0.27	0.35	0.48	-0.03
	LT22	-0.07	-0.73	0.00	0.13	-0.02	0.41	0.05
	CT2	0.12	-0.47	1.29	-0.19	0.32	—	0.18
	CT3	0.05	0.17	1.06	-0.41	0.05	—	-0.06
	CT8	0.44	0.14	1.08	0.02	-0.04	—	-0.11
25	CT10	0.35	0.26	1.60	-0.05	0.00	—	-0.02
	CT12	-0.15	-0.46	0.52	-0.13	0.02	—	-0.20
	CT14	0.26	-0.59	1.05	-0.01	0.68	—	0.48
	CT15	0.55	-0.51	1.36	-0.69	0.11	—	-0.16
	CT16	0.09	-0.14	1.06	0.00	0.00	—	-0.15
	CT17	0.40	-0.16	1.00	-0.47	0.04	—	-0.29

Table 18 indicates the  $\Delta C_t$  values for the indicated epicenter markers, indicating the relative amplification along chromosome 17 in the immediate vicinity of DNA52722.

Table 18  
Amplification of Epicenter Markers Relative to DNA52722

	tumor	Epicenter marker						
		Q33	120F17FO R1	120F17FO R2	DNA5272 2	120F17REV 1	120F17REV 2	Q34
5	LT1	-0.18	0.11	0.00	0.20	-0.08	0.07	-0.36
	LT1a	0.32	-0.06	0.00	0.68	-0.09	-0.20	0.32
	LT2	0.06	0.14	0.00	0.27	-0.29	0.16	-0.16
	LT3	0.08	-2.06	0.00	0.16	-0.84	-0.38	-0.16
	LT4	---	---	---	---	---	---	---
	LT6	---	---	---	---	---	---	---
10	LT7	-0.20	-0.51	0.00	0.23	-0.63	-0.37	-0.41
	LT9	0.08	-0.17	0.00	0.59	0.02	-0.66	-0.01
	LT10	0.09	0.05	0.00	0.59	-0.22	-0.12	0.36
	LT11	0.75	0.09	0.00	1.07	0.43	-0.01	0.63
	LT12	0.00	-0.45	0.00	0.63	-0.49	-0.82	0.18
	LT13	0.72	-0.02	0.00	1.29	0.04	0.02	0.66
15	LT15	0.75	0.11	0.00	1.33	0.15	-0.19	0.90
	LT16	0.34	-0.41	0.00	1.11	-0.39	-0.89	0.15
	LT17	1.06	0.29	0.00	1.13	-0.26	-0.12	0.90
	LT18	0.66	0.11	0.00	1.21	-0.28	0.11	0.47
	LT19	-0.09	-0.37	0.00	0.12	-0.53	-0.48	-0.53
	CT1	0.50	0.14	0.00	1.22	0.27	0.43	0.72
20	CT2	0.69	-0.47	0.00	0.95	-0.72	-0.17	0.77
	CT3	0.87	0.08	0	1.19	-0.06	0.74	0.97
	CT4	0.45	-0.11	0	1.26	0.43	0.38	0.79
	CT5	0.36	-0.39	0	1.79	-0.48	0.09	0.95
	CT6	0.41	0.08	0	1.71	-0.21	0.57	0.47
	CT7	0.40	0.18	0	1.19	0.31	0.40	0.54
25	CT8	0.48	0.17	0	0.93	0.23	0.47	0.72
	CT10	0.72	0.15	0	1.86	0.81	0.67	0.97
	CT11	0.80	-0.09	0	2.29	0.20	0.25	0.85
	CT12	0.01	-0.55	0	0.49	-0.43	-0.09	0.11
	CT14	0.22	-0.36	0	1.05	0.63	0.41	0.40
	CT15	1.06	-0.04	0	1.27	0.74	0.98	1.13
30	CT16	0.84	0.06	0	1.03	0.26	0.40	0.91

CT17	0.80	0.04	0	0.95	0.78	1.29	0.90
CT18	0.34	0.13	0	1.06	0.06	0.34	0.50

Tables 18A and 18B indicate the  $\Delta C_t$  values for the indicated epicenter markers, indicating the relative amplification of selected lung and colon tumors, respectively, along chromosome 17 in the immediate vicinity of DNA48227.

Table 18A

Amplification of Epicenter Markers in vicinity of DNA48227 on chromosome 17 in selected lung tumors

Tumor	Epicenter Marker							
	Q7 3	Q74	203J20FOR 1	203J20FOR 2	Q7 2	DNA4822 7	203J20REV 1	203J20REV 2
LT1	- 3.38	-0.07	-0.11	-0.83	- 0.37	0.15	-0.55	-0.08
LT1a	- 2.62	0.37	0.36	0.13	0.04	0.56	-0.13	0.30
LT2	- 1.56	0.26	0.24	-0.70	0.22	0.11	-0.23	0.14
LT3	- 0.01	0.05	0.31	-0.07	0.18	0.50	-0.34	0.23
LT4	- 4.58	-0.15	-0.16	-0.32	- 0.18	0.18	-0.26	0.29
LT6	- 0.76	-0.31	0.17	-0.32	- 0.16	0.07	-0.37	-0.10
LT7	- 2.10	0.17	0.18	-0.16	0.56	0.23	-0.13	0.24
LT9	- 2.10	0.03	0.07	-0.31	- 0.32	-0.07	-0.38	-0.02
LT10	- 3.17	0.22	0.40	-0.16	0.47	0.27	-0.30	-0.09
LT11	0.47	-0.01	0.34	0.29	0.96	0.18	0.47	0.15
LT12	0.44	0.32	0.10	0.13	1.24	0.20	0.36	0.17
LT13	0.18	0.03	-0.07	0.15	1.25	0.08	0.04	-0.03
LT15	0.00	0.11	0.00	0.09	1.72	0.17	0.06	-0.04
LT16	0.54	0.52	0.65	0.22	1.62	0.17	0.57	0.29
LT17	0.24	0.27	0.23	0.36	1.09	0.13	0.35	0.06
LT18	- 0.22	-0.26	0.12	0.21	- 0.11	-0.46	-0.26	-0.25

LT22	- 0.20	-0.32	0.02	0.11	- 0.12	-0.21	-0.24	-0.08
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Table 18B

Amplification of Epicenter Markers in vicinity of DNA48227 on chromosome 17 in selected colon tumors

Tumor	Epicenter Marker							
	Q7 4	203J20FOR 1	203J20FOR 2	Q7 2	DNA4822 7	203J20REV 1	203J20REV 2	Q75
CT1	- 0.18	0.38	0.19	0.07	1.09	0.31	0.04	0.36
CT2	0.49	0.20	0.64	0.39	2.10	0.38	0.20	0.26
CT3	0.18	0.22	0.51	0.43	1.09	0.38	-0.03	-0.22
CT4	0.27	0.25	0.39	- 0.14	1.12	0.07	-0.13	0.23
CT5	0.31	0.11	-0.18	- 0.83	2.33	0.03	0.10	0.27
CT6	0.34	0.04	0.01	0.02	1.35	0.07	0.60	0.63
CT7	0.02	-0.13	-0.16	- 0.07	0.56	-0.11	-0.15	0.50
CT8	- 0.08	0.08	0.17	0.15	1.34	0.11	-0.03	0.25
CT9	0.15	0.07	-0.02	0.44	1.22	-0.02	-0.06	0.52
CT10	- 0.14	0.18	0.19	0.56	1.66	0.29	0.10	0.17
CT11	0.13	-0.15	0.12	0.07	1.96	0.21	-0.07	0.57
CT12	0.00	-0.05	0.25	0.05	1.03	0.07	0.10	0.07
CT14	0.59	0.31	0.70	0.44	1.69	0.47	0.35	0.48
CT15	0.22	0.06	0.38	0.42	1.77	0.22	-0.05	0.27
CT16	- 0.69	-0.04	0.40	- 0.08	0.92	0.13	-0.02	-0.04
CT17	- 0.73	-0.08	-0.36	0.28	1.25	0.08	0.06	0.20
CT18	0.18	-0.22	-0.10	0.08	0.97	-0.05	0.18	0.63

PRO357:

PRO357 (UNQ324, DNA44804) was reexamined with selected tumors from the above initial  
 25 screen with framework mapping. Figure 40 and Table 19 indicate the chromosomal mapping of the

framework markers that were used in the present example. The framework markers are located approximately every 20 megabases and were used to control aneuploidy.

- PRO357 (UNQ324, DNA44804) was also examined with epicenter mapping. The markers indicated in Table 20 are located in close proximity (in the genome) to DNA44804 and are used to assess the relative amplification in the immediate vicinity of Chromosome 16 wherein DNA44804 is located. The distance between individual markers is measured in centirays (cR), which is a radiation breakage unit approximately equal to a 1% chance of a breakage between the two markers. One cR is very roughly equivalent to 20 kilobases. The marker SHGC-6154 is the marker found to be the closest to the location on chromosome 16 where DNA44804 maps.

10

Table 19  
Framework markers for DNA44804

Map position on chromosome 16	Stanford Human Genome Center Marker Name
P7	SHGC-2835
P55	SHGC-9643
P99	GATA7B02
P154	SHGC-33727
P208	SHGC-13577

15

Table 20  
Epicenter markers for DNA44804 along chromosome 16

Map position on chromosome 16	Stanford Human Genome Center Marker Name	Distance to next Marker (cR)
P1	AFMA139WG1	6
P3	SHGC-32420	170 (gap)
P4	SHGC-14817	40
P5	SHGC-12265	4
P6	SHGC-6154	33
DNA44804	—	—
P7	SHGC-2835	10
P8	SHGC-2850	9
P9	AFM297yg5	67
P15	CHLC.GATA70B04	—

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The  $\Delta C_t$  values of the above described framework markers along chromosome 16 relative to DNA44804 is described in Table 21.

Table 21

Amplification of Framework Markers relative to DNA44804 ( $\Delta C_t$ )

tumor	framework marker					
	DNA44804	P7	P55	P99	P154	208
LT1	0.25	0.22	-0.17	0.42	0.04	0.43
LT1a	0.90	0.09	-0.10	-0.38	0.29	0.93
LT2	-0.16	0.03	0.19	-0.18	0.18	0.54
LT3	1.15	0.68	0.57	-0.34	-0.03	0.86
LT4	0.19	0.58	0.36	-0.31	0.08	1.14
LT6	0.28	0.27	-0.11	-0.74	-0.13	0.22
LT7	0.58	0.63	0.14	0.82	0.09	-0.21
LT9	0.68	0.63	0.14	0.82	0.09	-0.21
LT10	1.21	0.52	0.40	-0.39	-0.15	0.77
LT11	1.71	-0.79	1.31	0.73	-0.08	0.90
LT12	1.96	-0.95	0.94	0.00	-0.63	0.18
LT13	2.32	-0.97	0.94	0.88	-0.04	0.70
LT15	3.01	-0.54	0.60	0.12	0.14	1.15
LT16	0.67	-0.27	0.57	-0.39	0.08	1.04
LT17	1.64	0.25	1.10	0.28	0.10	0.23
LT18	0.34	0.09	0.51	0.33	-0.20	-0.09
LT19	3.03	-0.82	0.63	0.06	0.09	0.55
LT21	1.33	-1.19	1.01	0.11	0.34	0.07

Table 22 indicates the  $\Delta C_t$  values for the results of epicenter mapping relative to DNA44804, indicating the relative amplification in the region more immediate to the actual location of DNA44804 along chromosome 16.

Table 22  
Amplification of epicenter markers relative to DNA44804

Tumor	Epicenter marker									
	P1	P3	P4	P5	P6	DNA44804	P7	P8	P9	P15
LT1	0.31	-0.30	0.65	0.05	-0.33	0.16	-0.41	0.20	0.1	0.17
LT1a	-0.23	-17.67	0.97	-0.65	-1.83	0.56	-0.65	-0.28	-0.27	-0.07
LT2	0.18	-0.06	0.33	-0.11	-0.38	-0.32	-1.08	-0.31	-0.53	-0.05

	LT3	0.00	0.25	1.07	-0.23	-0.11	0.70	-0.71	-0.12	-0.17	-0.01
	LT4	0.07	-0.25	0.55	-1.15	-1.78	-0.09	-0.82	-0.07	-0.34	-0.07
	LT6	0.24	0.07	0.48	-0.55	-0.34	-0.07	-1.33	-0.41	-0.7	-0.27
	LT7	0.07	-0.07	0.61	-0.19	-0.36	0.29	-0.96	-0.09	-0.26	-0.08
5	LT9	0.16	-0.16	0.64	-0.33	-0.14	0.43	-1.01	-0.19	-0.36	-0.21
	LT10	0.47	0.76	-0.30	0.80	-0.09	0.00	-0.85	-0.17	-0.28	-0.07
	LT11	0.14	0.14	0.96	-0.02	0.37	1.27	-0.23	0.09	-0.33	-0.07
	LT12	-0.12	-0.04	0.84	-1.52	-0.28	1.42	-0.39	-0.38	-1.21	-0.25
	LT13	0.41	-0.02	1.19	-0.34	0.14	1.67	-0.87	-0.22	-0.72	-0.33
10	LT15	0.01	0.21	1.30	-0.48	-0.35	2.36	-0.96	-0.36	-0.54	-0.22
	LT16	-0.38	-0.07	0.41	-0.32	-1.22	-0.08	-0.45	-0.25	-0.52	-0.31
	LT17	0.36	0.23	1.39	-1.39	01.37	1.17	-0.39	-0.13	0.52	0.01
	LT18	0.17	-0.27	0.04	-0.04	0.18	-0.39	-0.59	-0.25	-0.21	-0.22
	LT19	0.11	-0.02	1.27	-0.12	1.27	2.49	-0.30	-0.36	-0.82	-0.40
15	LT21	0.28	-0.18	0.85	0.09	0.66	0.85	-0.49	-0.35	-0.27	-0.16

#### DISCUSSION and CONCLUSION:

##### PRO201:

The  $\Delta C_t$  values for DNA30676 (PRO201, UNQ175) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA30676 occurred in primary lung tumors: SRC727 (LT3), SRC732 (LT10), SRC733 (LT11), SRC735 (LT13), SRC736 (LT15), SRC737 (LT16), SRC738 (LT17), SRC740 (LT19), SRC741 (LT21); primary colon tumors SRC742 (CT2), SRC743 (CT3), SRC744 (CT8), SRC745 (CT10), SRC746 (CT12), SRC747 (CT14), SRC748 (CT15), SRC749 (CT16), SRC750 (CT17), SRC751 (CT1), SRC752 (CT4), SRC753 (CT5), SRC754 (CT6), SRC755 (CT7), SRC756 (CT9), SRC757 (CT11), SRC758 (CT18); lung tumor cell lines SRC769 (Calu-1), SRC770 (Calu-6), SRC771 (H157), SRC772 (H441), SRC774 (SKEMS-1), SRC832 (H522), SRC833 (H810); and colon tumor cell lines SRC778 (Colo320), SRC828 (Colo205).

The  $\Delta C_t$  values and average  $\Delta C_t$  values for the primary lung tumors hits are: 2.05, 1.08, 1.63, 2.13, 2.58, 1.10, 1.63, 3.37, 1.3; primary colon tumors are: 3.81, 2.3, 1.97, 3.01, 2.46, 3.34, 2.58, 2.46, 2.64, 1.57, 3.39, 2.95, 2.81, 2.77, 2.63, 3, 2.23; lung tumor cell lines are 2.37, 2.61, 2.39, 2.07, 2.91, 2.07; colon tumor cell lines 1.95, 1.26. This represents an increase in gene copy of approximately 4.14, 2.11, 3.10, 4.38, 5.98, 2.14, 3.10, 10.34, 2.46 for the primary lung tumors, 14.03, 4.92, 3.92, 8.06, 5.50, 10.13, 5.98, 5.50, 6.23, 2.97, 10.48, 7.73, 7.01, 6.82, 6.19, 8.0, 4.69 for the primary colon tumors, 5.17, 6.10, 5.24, 6.19, 7.52, 4.20 for the lung tumor cell lines, and 3.86, 2.39 for the colon tumor cell lines.

Amplification has been confirmed by framework mapping for DNA30676: in primary lung tumors LT3, LT15-LT18; and in primary colon tumors CT2, CT3, CT8, CT10, CT12, CT14, CT15,



CT16, CT17. The reported  $\Delta C_t$  values for the primary lung tumors are 1.04, 1.43, 1.35, 1.51 and 1.22, while the primary colon tumors report 2.81, 2.03, 1.39, 2.21, 1.93, 2.37, 1.27, 1.76 and 1.65. Relative to normal tissue, this represents approximately a 2.06, 2.69, 2.55, 2.85, and 2.32 fold increase for the lung tumors, and a 7.01, 4.08, 2.62, 4.63, 3.81, 5.17, 2.41, 3.39, 3.14 fold increase for the colon tumors.

- 5 Epicenter mapping for DNA30676 resulted in confirmation of significant amplification: in primary lung tumors LT3, LT13, LT15, LT16, LT18; and in primary colon tumors CT1, CT3, CT4, CT5, CT6, CT7, CT8, CT9, CT10, CT11, CT12, CT14, CT15, CT16, CT17 and CT18. The reported  $\Delta C_t$  values for the primary lung tumors were 1.87, 1.44, 1.99, 1.72 and 1.09, while the primary colon tumors indicated  $\Delta C_t$  and average  $\Delta C_t$  values of 2.56, 2.19, 3.22, 2.83, 2.93, 2.43, 1.95, 2.72, 3.12, 3.26, 2.77, 2.88, 2.60, 2.61, 10 2.24 and 2.48. Relative to normal tissue, this represents a 3.66, 2.71, 3.97, 3.29, 2.13-fold increase in gene copy for the lung tumors and a 5.90, 4.56, 9.32, 7.11, 7.62, 5.39, 3.86, 6.59, 8.69, 9.58, 6.82, 7.36, 6.06, 6.11, 4.72, 5.58-fold increase in gene copy for the colon tumors.

In contrast, the amplification of the closest known framework markers (with one exception, *i.e.* S50)(Table 5A) or epicenter markers (Table 6) does not occur to a greater extent than that of DNA30676.

- 15 This strongly suggests that DNA30676 is the gene responsible for the amplification of the particular region on Chromosome 19. Because amplification of DNA30676 occurs in various lung and colon tumors and cell lines (especially colon), it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (*e.g.*, antibodies) directed against the protein encoded by DNA30676 (PRO201) would be expected to have utility in cancer therapy.

#### 20 PRO292:

The  $\Delta C_t$  values for DNA35617 (PRO292, UNQ266) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  value of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA35617 occurred in primary lung tumors: LT12, LT13, LT15, LT19, LT21; in primary colon tumors CT2, CT8, 25 CT10, CT14; in lung tumor cell lines H441 and H810; in colon tumor cell lines SW620, Colo320, HT29 and LS174T. The  $\Delta C_t$  and average  $\Delta C_t$  values for these hits are: (1) primary lung tumors: 1.35, 1.93, 2.15, 2.33, 1.42; (2) primary colon tumors: 3.54, 1.52, 1.63, 1.58; (3) lung tumor cell lines: 1.13, 1.08; and (4) colon tumor cell lines: 1.41, 1.34, 1.10, 1.50. Relative to normal tissue, this represents approximately a 2.55, 3.81, 4.44, 5.03, 2.67-fold increase, respectively, in gene copy for the primary lung tumor hits, a 30 11.63, 2.87, 3.09, 2.99-fold increase, respectively, in gene copy for the primary colon tumors, a 2.19 and 2.11-fold increase in gene copy for the lung tumor cell lines, and a 2.66, 3.53, 2.14 and 2.83-fold increase in gene copy for the colon tumor cell lines.

- Amplification of DNA35617 was also confirmed by framework mapping. Table 8 indicates that significant amplification of DNA35617 was confirmed in LT12, LT13, LT15, LT16 and CT2, CT8, CT10 35 and CT14. The reported  $\Delta C_t$  values for the primary tumors were 1.61, 1.95, 2.86 and 1.68, while for the primary colon tumors the values were 3.75, 1.76, 1.68 and 1.75. These represent a 3.05, 3.86, 7.26, 3.20-fold increase in gene copy for the lung tumors and a 13.45, 3.39, 3.20 and 3.36-fold increase in gene copy for the colon tumors.

In contrast, the amplification of the closest known framework markers (Table 8) does not occur to a greater extent than that of DNA35617. This strongly suggests that DNA35617 is the gene responsible for the amplification of the particular region on chromosome 17. Because amplification of DNA35617 occurs in various tumor tissues, especially colon tumors, it is highly probable to play a significant role in tumor formation or growth, in particular, colon tumor formation and growth. As a result, antagonist (e.g., antibodies) directed against the protein encoded by DNA35617 would be expected to have utility in cancer therapy.

#### PRO327:

The  $\Delta C_t$  values for DNA38113 (PRO327)(UNQ288) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA38113 occurred in primary lung tumors: SRC734 (LT12), SRC735 (LT13), SRC736 (LT15), SRC737 (LT16), SRC738 (LT17), SRC740 (LT19); primary colon tumors: SRC742 (CT2), SRC743 (CT3), SRC744 (CT8), SRC745 (CT10), SRC746 (CT12), SRC747 (CT14), SRC748 (CT15), SRC749 (CT16), SRC750 (CT17), SRC751 (CT1), SRC752 (CT4), SRC753 (CT5), SRC754 (CT6), SRC756 (CT9), SRC757 (CT11), SRC758 (CT18); lung tumor cell lines SRC771 (H157), SRC772 (H441), SRC773 (H460), SRC774 (SKMES-1), SRC832 (H522), SRC833 (H810); colon tumor cell lines: SRC777 (SW620), SRC778 (Colo320), SRC830 (HCC2998), SRC831 (KM12).

The  $\Delta C_t$  values and average  $\Delta C_t$  values for the primary lung tumor hits are: 2.57, 2.09, 3.44, 1.91, 1.43, 3.58; primary colon tumors are: 3.64, 1.12, 1.64, 2.53, 1.88, 2.51, 1.30, 1.47, 1.71, 1.37, 2.22, 2.50, 2.28, 1.58, 3.33 and 1.07; lung tumor cell lines 1.65, 2.23, 1.12, 1.18, 1.02 and 1.1; and colon tumor cell lines 2.24, 1.01, 1.23 and 1.61. This represents an increase in gene copy of approximately: 5.94, 4.26, 10.85, 3.75, 2.69, 11.96 for the primary lung tumors; 12.47, 2.17, 3.12, 5.78, 3.68, 5.67, 2.46, 2.77, 3.27, 2.58, 4.66, 5.66, 4.86, 2.99, 10.06, 2.10 for the primary colon tumors; 3.14, 4.69, 2.17, 2.27, 2.03, 2.14 for the lung tumor cell lines, and 4.73, 2.01, 2.35 and 3.05 for the colon tumor cell lines.

Amplification has been confirmed by framework mapping for DNA38113 in primary lung tumors LT10 and in primary colon tumors CT2, CT3, CT8, CT10, CT12, CT14, CT16. The reported  $\Delta C_t$  values for the primary lung tumor is 1.45; the primary colon tumors are 2.94, 1.23, 1.45, 1.72, 1.60, 2.03 and 1.07. Relative to normal tissue, this represents approximately a 2.73-fold increase for the lung tumor and a 7.67, 2.34, 2.73, 3.29, 3.03, 4.08 and 2.10-fold increase for the colon tumors. Epicenter mapping resulted in confirmation of significant amplification in: primary lung tumors LT12, LT13, LT15, LT16, LT17 and in primary colon tumors CT1, CT2, CT3, CT4, CT5, CT6, CT8, CT9, CT10, CT11, CT12, CT14, CT16. The reported  $\Delta C_t$  values for the primary lung tumors are: 1.57, 1.22, 2.47, 2.23, 1.02 and for the primary colon tumors are 1.38, 2.94, 1.23, 2.23, 2.51, 2.29, 1.45, 1.59, 1.72, 3.34, 1.6, 2.03, 1.07 and 1.08. Relative to normal tissue, this represents a 2.97, 2.33, 5.54, 4.69, 2.03-fold increase in gene copy for the lung tumors and a 2.60, 7.67, 2.35, 4.69, 5.70, 4.89, 2.73, 3.01, 3.29, 10.13, 3.03, 4.08, 2.10, 2.11-fold increase in gene copy for the colon tumors.

With the exception of S41, amplification of the closest markers to DNA38113 does not occur to a greater extent than that of DNA38113 itself. This supports that notion that DNA38113 is the gene which is driving the amplification of this particular region of Chromosome 19. However, the amplification of marker S41 (which does not map closely to DNA38113) could be an independent amplification event or even an error in the ordering of the markers. Because amplification of DNA38113 occurs in various lung and colon tumors and cell lines, it is highly probably to play a significant role in tumor formation and growth. As a result, antagonists (e.g., antibodies) directed against the protein encoded by DNA38113 (PRO327) would be expected to have utility in cancer therapy.

#### PRO1265:

The  $\Delta C_t$  values for DNA60764 (PRO1265)(UNQ636) in a variety of lung tumors are reported in Table 2. A  $\Delta C_t$  value of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA60765 occurred in primary lung tumors LT3, LT12, LT13, LT15, LT16 and LT17. The  $\Delta C_t$  values of these hits are 1.03, 2.17, 2.24, 3.51, 3.32 and 1.02. This represents an increase in gene copy of approximately 2.04, 4.50, 4.72, 11.39, 9.99 and 2.03.

Amplification has also been confirmed framework mapping for DNA60764 in LT16. The reported  $\Delta C_t$  value was 1.37, which represents a 2.58 fold increase in gene copy relative to normal tissue. Epicenter mapping has also confirmed amplification of DNA60764 in LT12, LT13, LT15, LT16, CT1, CT4, CT5, CT7 and CT11. These tumors report  $\Delta C_t$  values of 2.35, 2.37, 3.88, 3.32 in the lung tumors and 1.74, 1.86, 3.28, 1.29 and 2.32 in the colon tumors. Relative to normal tissue, this represents an increase in gene copy of approximately 5.10, 5.17, 14.72 and 9.98 in the lung tumors and 3.34, 3.63, 9.71, 2.45 and 4.99 in the colon tumors.

In contrast, the amplification of the closest known framework markers, epicenter markers and the comparison sequences does not occur to a greater extent than that of DNA60764. This strongly suggests that DNA60764 is the gene responsible for the amplification of the particular region in Chromosome 19. Because amplification of DNA60764 occurs in various lung and colon tumors, it is highly probably to play a significant role in tumor formation or growth. As a result, antagonists (e.g., antibodies) directed against the protein encoded by DNA60764 would be expected to have utility in cancer therapy.

#### PRO344:

The  $\Delta C_t$  values for DNA40592 (PRO344, UNQ303) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA40592 occurred in primary lung tumors LT13 and LT19, in primary colon tumors CT2, CT14, CT15, CT1, CT4, CT5, CT11. The  $\Delta C_t$  values and average  $\Delta C_t$  of these hits are 1.34, 1.52 in the lung tumors, and 1.84, 1.16, 1.17, 1.24, 1.46, 1.51 and 1.35. This represents approximately a 2.53, 2.87 and 3.58, 2.23, 2.25, 2.36, 2.75, 2.85, 2.5-fold increase, respectively, in gene copy relative to normal tissue. Because amplification of DNA40592 occurs in various tumors, it is likely associated with tumor formation or growth. As a result,

antagonists, (e.g., antibodies) directed against the protein encoded by DNA40592 (PRO344) would be expected to be useful in cancer therapy.

PRO343:

The  $\Delta C_t$  values for DNA43318 (PRO343)(UNQ302) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA43318 occurred in primary lung tumor: LT13, LT15, LT19, CT2, CT8, CT10, CT12, CT14, CT15, CT16, CT17 and CT5. The  $\Delta C_t$  and average  $\Delta C_t$  values for the primary lung tumor hits are 1.67, 1.47 and 1.66, while the colon tumor hits are 2.72, 1.43, 1.68, 1.33, 2.02, 1.73, 1.16, 1.5, 1.34. This represents an increase in gene copy of approximately 3.18, 2.77 and 3.16 -fold for the lung tumors and 6.59, 2.69, 3.20, 2.51, 4.06, 3.31, 2.23, 2.83 and 2.53 -fold for the colon tumors.

Amplification has been confirmed by framework mapping for DNA43318: in primary lung tumors LT12, LT13, LT15, LT16 and LT18; and in primary colon tumors CT2, CT4, CT5, CT8, CT10, CT14, CT15 and CT16. The reported  $\Delta C_t$  values are 1.16, 1.72, 2.73, 1.46 and 1.06 for the lung tumors and 3.54, 1.17, 2.63, 1.44, 1.36, 1.68, 1.07 and 1.01 for the colon tumors. Relative to normal tissue, this represents about a 2.23, 3.29, 6.63, 2.75 and 2.08-fold increase in gene copy for the lung tumors, and about a 11.63, 2.25, 6.19, 2.71, 2.57, 3.20, 2.10 and 2.01-fold increase for the colon tumors. Epicenter mapping for DNA43318 confirmed significant amplification in LT12, LT13, LT15, LT16, CT4, CT5, CT6, CT11 and CT2. The reported  $\Delta C_t$  values are 1.32, 1.94, 3.07, 1.83, 1.02, 2.40, 3.78, 1.51 and 2.48. Relative to normal tissue, this represents a 2.50, 3.84, 8.40, 3.56-fold increase in the lung tumors and a 2.03, 5.28, 13.74, 2.85, 5.58-fold increase in gene copy for the colon tumors.

In contrast, the amplification of the closest known framework and epicenter markers (with one exception, i.e. P107, Table 10.1) does not occur to a greater extent than that of DNA43318. This strongly suggests that DNA43318 is the gene responsible for the amplification of the particular region of Chromosome 16. Because amplification of DNA43318 occurs in various lung and colon tumors, it is highly probably to play a significant role in tumor formation or growth. As a result, antagonists (e.g., antibodies) directed against the protein encoded by DNA43318 would be expected to have utility in cancer therapy.

PRO347:

The  $\Delta C_t$  values for DNA44176 (PRO347, UNQ306) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA44176 occurred in primary lung tumors LT15 and primary colon tumors CT2, CT8, CT14, CT15, CT16, CT5 and CT11. The  $\Delta C_t$  values and average  $\Delta C_t$  of these hits are 1.76 in the lung tumors, and 1.85, 1.12, 1.27, 1.07, 1.19, 1.80, 1.42 in the

colon tumors. This represents approximately a 3.39, 3.61, 2.17, 2.41, 2.10, 2.28, 3.48, 2.67-fold increase, respectively, in gene copy relative to normal tissue.

Because amplification of DNA44176 occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists, (e.g., antibodies) directed against the protein encoded by  
 5 DNA44176 (PRO347) would be expected to be useful in cancer therapy.

#### PRO357:

The  $\Delta C_t$  values for DNA44804 (PRO357)(UNQ314) in a variety of lung and colon tumor and cell lines are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of  
 10 DNA44804 occurred in primary lung tumors: LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT17, LT19, LT21; in primary colon tumors CT2, CT8, CT10, CT14, CT15, CT16, CT1, CT4, CT5, CT6, CT7 and CT11. The  $\Delta C_t$  and average  $\Delta C_t$  values of the lung tumor hits are: 1.42, 1.63, 1.47, 1.42, 1.52, 2.18, 1.23, 1.71, 2.47, 1.66; and the colon tumor hits are 2.01, 1.1, 1.42, 1.5, 1.25, 1.05, 1.04, 1.27, 1.69, 1.08, 1.73, 1.83. This represents an increase in gene copy of approximately 2.68, 3.09, 2.77, 2.68, 2.87, 4.53, 2.36,  
 15 3.27, 5.54 and 3.1-fold, respectively, for the lung tumors and a 4.03, 2.14, 2.68, 2.83, 2.38, 2.07, 2.06, 2.41, 3.23, 2.11, 3.31, 3.56-fold increase, respectively, for the colon tumors.

Amplification has been confirmed by framework mapping for DNA44804 in primary lung tumors LT3, LT10, LT11, LT13, LT15, LT17, LT19 and LT21. The reported  $\Delta C_t$  values for these hits are 1.15, 1.21, 1.71, 1.96, 2.32, 3.01, 1.64, 3.03 and 1.33. These represent a 2.22, 2.31, 3.27, 3.89, 4.99, 8.06, 3.12,  
 20 8.17 and 2.51-fold increase in gene copy in the respective primary lung tumors. Epicenter mapping for DNA44805 resulted in confirmation of significant amplification in primary lung tumors 1.27, 1.42, 1.67, 2.36, 1.17 and 2.29, which represents an increase in gene copy of 2.41, 2.68, 3.18, 5.13, 2.25, 4.89-fold, respectively.

In contrast, the amplification of the closest known framework markers (from Table 21) and  
 25 epicenter markers (Table 22) does not occur to a greater extent than that of DNA44804. This strongly suggests that DNA44804 is the gene responsible for the amplification of the particular region on chromosome 16. Because amplification of DNA44804 occurs in various tumor tissues, especially colon tumors, it is highly probable to play a significant role in tumor formation or growth, in particular, colon tumor formation and growth. As a result, antagonist (e.g., antibodies) directed against the protein encoded  
 30 by DNA44804 would be expected to have utility in cancer therapy.

#### PRO715:

The  $\Delta C_t$  values for DNA52722 (PRO715)(UNQ383) in a variety of lung and colon tumor and cell lines are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification  
 35 scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA52722 occurred in LT12, LT13, LT15, LT17, LT19, CT2, CT3, CT8, CT10, CT14, CT15, CT16, CT17, CT1, CT4, CT5, CT6, CT7, CT11, CT18. The  $\Delta C_t$  and average  $\Delta C_t$  values for the primary lung tumors are 1.29, 1.51, 1.35, 1.22 and 1.55, while the primary colon tumors report values of 1.64, 1.12,

1.32, 1.97, 1.42, 1.53, 1.28, 1.31, 1.32, 1.59, 1.92, 1.43, 1.23, 2.29, 1.05. Relative to normal tissue, these represent 2.45, 2.85, 2.55, 2.33, 2.93-fold increase in gene copy for the lung tumors and 3.12, 2.17, 2.50, 3.92, 2.68, 2.89, 2.42, 2.48, 2.50, 3.01, 3.78, 2.69, 2.34, 4.89, 2.07-fold increase in gene copy for the colon tumors.

- 5 Significant amplification has been confirmed by framework mapping for DNA52722: in primary lung tumors LT11, LT13, LT15, LT16, LT17 and LT18 and in primary colon tumors CT2, CT3, CT8, CT10, CT14, CT15 and CT16. The reported  $\Delta C_t$  values for the lung tumors are 1.03, 1.37, 1.75, 1.11, 1.14, 1.04 and for the colon tumors 1.29, 1.06, 1.08, 1.60, 1.05, 1.36 and 1.06. Relative to normal tissue, the represents approximately a 2.04, 2.58, 3.36, 2.16, 2.20, 2.06 -fold increase in gene copy for the lung
- 10 tumors and a 2.44, 2.08, 2.11, 3.03, 2.07, 2.57 and 2.08-fold increase in gene copy for the colon tumors. Significant amplification was also confirmed by epicenter mapping for DNA52722, wherein hits were observed in LT11, LT13, LT15, LT16, LT17, LT18, CT1, CT3, CT4, CT5, CT6, CT7, CT10, CT11, CT14, CT15, CT16 and CT18. The reported  $\Delta C_t$  values for the primary lung tumors were 1.07, 1.29, 1.33, 1.11, 1.13, 1.21, while the colon tumors indicate 1.22, 1.19, 1.26, 1.79, 1.71, 1.19, 1.86, 2.29, 1.05, 1.27,
- 15 1.03 and 1.06. Relative to normal tissue, this represents a 2.10, 2.45, 2.51, 2.16, 2.19, 2.31-fold increase in the lung tumors and a 2.33, 2.28, 2.39, 3.46, 3.27, 2.28, 3.63, 4.89, 2.07, 2.41, 2.04 and 2.08-fold increase in the colon tumors.

- In marked contrast, the amplification of the closest known framework and epicenter markers does not occur to a great extent than that of DNA52722. This strongly suggests that DNA52722 is the gene
- 20 responsible for the amplification of the particular region on Chromosome 17. Because amplification of DNA52722 occurs in various lung and colon tumors and cell lines, it is highly probable to play a significant role in tumor formation or growth. As a result, antagonists (e.g., antibodies) directed against the protein encoded by DNA52722 (PRO715) would be expected to have utility in cancer therapy.

#### PRO1017:

- 25 The  $\Delta C_t$  values for DNA56112 (PRO1017, UNQ500) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  value of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA56112 occurred in: primary lung tumors: LT1a, LT3, LT6, LT7, LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT17, LT18, LT19, LT21; primary colon tumors: CT2, CT3, CT8, CT10, CT12, CT14, CT15, CT16,
- 30 CT17, CT4, CT5, CT6, CT9, CT11. The  $\Delta C_t$  and average  $\Delta C_t$  values for (1) the primary lung tumor hits are: 1.78, 2.37, 2.79, 1.11, 2.73, 3.51, 1.96, 2.20, 1.90, 2.24, 1.78, 1.76, 1.24 and 2.24; and (2) primary colon tumor hits are: 3.13, 2.74, 1.78, 2.67, 2.29, 2.87, 2.30, 1.75, 1.82, 1.10, 2.20, 1.11, 1.28 and 1.47. This represents an increase in gene copy of approximately 3.43, 5.17, 6.92, 2.16, 6.63, 11.39, 3.89, 4.59, 3.73, 4.72, 3.43, 3.39, 2.36 and 4.72 -fold, respectively, for the lung colon tumors and 8.75, 6.68, 3.43,
- 35 6.35, 4.89, 7.31, 4.92, 3.36, 3.53, 2.14, 4.59, 2.16, 2.43 and 2.77-fold, respectively, for the primary colon tumors.

Amplification has also been confirmed by framework mapping for DNA56112 in primary lung tumors LT3, LT4, LT7, LT9, LT10, LT11, LT12, LT13, LT15, LT16, LT18 and LT22. The reported  $\Delta C_t$  values are 1.21, 1.59, 1.40, 1.07, 1.98, 1.15, 2.31, 1.83, 2.79, 2.22, 1.06 and 1.18. These values represent a 2.31, 3.01, 2.64, 2.10, 3.95, 2.22, 4.96, 3.56, 6.92, 4.66, 2.08 and 2.27-fold increase, respectively, in gene copy for the indicated lung tumors. Epicenter mapping for DNA56112 indicated in confirmation of amplification in: primary lung tumors: LT12, LT13, LT15, LT16 and LT18 and in primary colon tumors: CT5, CT8, CT10, CT12, CT14, CT16 and CT17. The  $\Delta C_t$  values for the primary lung tumors are: 1.47, 1.51, 2.27, 1.62 and 1.03; and the primary colon tumors are 1.29, 1.46, 2.28, 1.84, 2.56, 1.22 and 1.52. These values represent a 2.77, 2.85, 4.82, 3.07, 2.04-fold increase, respectively, in the lung tumors and a 2.45, 2.75, 4.86, 3.58, 5.90, 2.33 and 2.87-fold increase, respectively, in the colon tumors.

In contrast, the amplification of the closest known framework markers (from Table 12) and epicenter markers (Table 11.2) does not occur to a greater extent than that of DNA56112. This strongly suggests that DNA56112 is the gene responsible for the amplification of the particular region on chromosome 17. Because amplification of DNA56112 occurs in various tumor tissues, especially colon tumors, it is highly probably to play a significant role in tumor formation or growth, in particular, colon tumor formation and growth. As a result, antagonist (e.g., antibodies) directed against the protein encoded by DNA56112 would be expected to have utility in cancer therapy.

#### PRO1112:

The  $\Delta C_t$  values for DNA57702 (PRO1112, UNQ555) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA57702 occurred in lung tumors LT10, LT11, LT12, LT13, LT15, LT17, LT18 and in colon tumors CT2, CT8, CT10, CT12, CT14, CT15, CT16, CT1, CT4, CT5, CT6 and CT11.

The  $\Delta C_t$  values and average  $\Delta C_t$  of these hits are 1.13, 1.52, 1.19, 1.63, 1.77, 1.45 and 1.25 for the lung tumor and 2.26, 1.06, 1.57, 1.31, 1.89, 1.46, 1.25, 1.24, 1.53, 1.97, 1.57 and 2.28 for the colon tumors. This represents approximately a 2.19, 2.87, 2.28, 3.09, 3.41, 2.73, 2.38-fold amplification, respectively, for the lung tumor, and 4.79, 2.08, 1.57, 2.97, 3.70, 2.75, 2.38, 2.36, 2.89, 3.92, 2.96, 4.5-fold amplification, respectively, for the colon tumors, in gene copy relative to normal tissue.

Because amplification of DNA57702 occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists, (e.g., antibodies) directed against the protein encoded by DNA57702 (PRO1112) would be expected to be useful in cancer therapy.

#### PRO509:

The  $\Delta C_t$  values for DNA50148 (PRO509, UNQ329) in a variety of lung and colon tumors are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA50148 occurred in primary colon tumors CT15, CT17, CT6, CT11 and CT18. The  $\Delta C_t$  values and average  $\Delta C_t$

of these hits are 1.16, 1.02, 1.01, 1.34, 1.35, which represents a 2.23, 2.03, 2.01, 2.53, 2.55-fold increase, respectively, in gene copy relative to normal tissue.

Because amplification of DNA50148 occurs in various tumors, it is likely associated with tumor formation or growth. As a result, antagonists, (e.g., antibodies) directed against the protein encoded by DNA50148 (PRO509) would be expected to be useful in cancer therapy.

#### PRO853:

The  $\Delta C_t$  values for DNA48227 (PRO853)(UNQ419) in a variety of lung and colon tumors and cell lines are reported in Table 2. A  $\Delta C_t$  of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA48227 occurred in primary lung tumors LT12 and lung cell line H522, as well as in primary colon tumors CT2, CT3, CT8, CT10, CT12, CT14, CT15, CT16, CT17, CT1, CT4, CT5, CT7 and CT11. The  $\Delta C_t$  and average  $\Delta C_t$  values for these hits are 1.03, 1.02, 2.4, 1.52, 1.55, 1.97, 1.36, 1.75, 1.75, 1.11, 1.11, 1.09, 1.16, 1.95, 1.14, 1.88. This represents an increase in gene copy of approximately 2.04 for the primary lung tumor LT12, 2.03 for the lung tumor cell line H522 and 5.28, 2.87, 2.93, 3.92, 2.57, 3.36, 3.36, 2.16, 2.16, 2.13, 2.23, 3.86, 2.20, 3.68 for the primary colon tumors.

Confirmation of amplification was not confirmed in epicenter mapping for DNA48227 for primary lung tumors, but was seen in primary colon tumors CT1, CT2, CT3, CT4, CT5, CT6, CT7, CT8, CT9, CT10, CT11, CT12, CT14, CT15, CT17 and CT18. The reported  $\Delta C_t$  values in Table 18B are 1.09, 2.10, 1.09, 1.12, 2.33, 1.35, 1.34, 1.22, 1.66, 1.96, 1.03, 1.69, 1.77 and 1.25. This represents a 2.13, 4.29, 2.13, 2.17, 5.03, 2.55, 2.53, 2.33, 3.16, 3.89, 2.04, 3.23, 3.41 and 2.38 -fold amplification, relative to normal tissue, for these tumors.

In contrast, the amplification of the closest known framework markers (from Table 16) and epicenter markers (Table 18B) does not occur to a greater extent than that of DNA48227. This strongly suggests that DNA48227 is the gene responsible for the amplification of the particular region on chromosome 17. Because amplification of DNA47227 occurs in various tumor tissues, especially colon tumors, it is highly probable to play a significant role in tumor formation or growth, in particular, colon tumor formation and growth. As a result, antagonist (e.g., antibodies) directed against the protein encoded by DNA48227 would be expected to have utility in cancer therapy.

#### PRO882:

The  $\Delta C_t$  value for DNA58125 (DNA882, UNQ448) in a variety of lung and colon tumors and cell lines is reported in Table 2. A  $\Delta C_t$  value of  $>1$  was typically used as the threshold value for amplification scoring, as this represents a doubling of gene copy. Table 2 indicates that significant amplification of DNA58125 occurred in primary lung tumors: LT1a, LT3, LT6, LT9, LT10, LT11, LT12, LT13, LT15, LT17, LT19; primary colon tumors: CT2, CT3, CT8, CT10, CT12, CT14, CT15, CT16, CT1, CT4, CT5, CT11; lung tumor cell lines: H441; and colon tumor cell lines: SW620, Colo320, HT29, SKCO1, SW403, LS147T, Colo205, HCT15, HCC2998 and KM12. The  $\Delta C_t$  and average  $\Delta C_t$  values for the primary lung tumor hits are: 1.18, 1.26, 1.10, 1.81, 1.95, 1.29, 1.07, 1.36, 1.58, 1.45, 1.87; the colon



tumor hits report: 2.18, 1.64, 1.10, 1.64, 1.05, 1.62, 1.25, 1.29, 1.17, 1.1, 2.03, 2.13; lung tumor cell lines: 1.08; and colon tumor cell lines: 1.61, 1.25, 1.54, 1.70, 2.00, 1.19, 1.66, 1.41, 1.19, 1.32. This represents an amplification in gene copy of the following: (1) Primary lung tumors: 2.27, 2.39, 2.14, 3.51, 3.86, 2.44, 2.10, 2.57, 2.99, 2.73 and 3.65 -fold increase, respectively; (2) Primary colon tumors: 4.53, 3.12, 2.14, 3.12, 2.07, 3.07, 2.38, 2.45, 2.25, 2.14, 4.08, 2.50 -fold increase, respectively; (3) lung tumor cell line: 2.11-fold increase; and (4) colon tumor cell line: 3.05, 2.38, 2.91, 3.25, 4.0, 2.28, 3.16, 2.66, 2.28, 2.50-fold increase, respectively.

Amplification has been confirmed by framework mapping for DNA58125 in primary lung tumors LT3, LT13, LT15, LT17, LT18, CT1, CT2, CT3, CT4, CT5, CT6, CT8, CT10, CT12, CT14, CT15, CT18. The reported  $\Delta C_t$  values for the primary lung tumors are 1.02, 1.33, 1.83, 1.03 and 1.08, while the primary colon tumors values are 1.08, 2.27, 1.34, 1.13, 2.17, 1.41, 1.23, 1.74, 1.13, 1.74, 1.30 and 1.04. Relative to normal tissue, this represents approximately a 2.03, 2.51, 3.56, 2.04, 2.11-fold increase, respectively, for the lung tumors, and a 2.11, 4.82, 2.53, 2.19, 4.50, 2.66, 2.34, 3.34, 2.19, 3.34, 2.46 and 2.06-fold increase, respectively, for the colon tumors.

Epicenter mapping further confirmed significant amplification in primary colon tumors LT12, LT13, LT15, LT16, LT17 and in primary colon tumors CT1, CT4, CT6, CT7, CT9, CT11, CT2, CT8, CT10 and CT16. The reported  $\Delta C_t$  values in the primary lung tumors are 1.02, 1.52, 2.04, 1.09 and 1.32, while the colon tumor values are 2.29, 1.49, 1.83, 1.20, 1.67, 1.02, 1.07, 2.27, 1.50 and 2.24. Relative to normal tissue, this represents approximately a 2.03, 2.87, 4.11, 2.13, 2.50-fold increase, respectively, in gene copy for the lung tumors and a 4.89, 2.81, 3.56, 2.30, 3.18, 2.03, 2.10, 4.82, 2.83, 4.72-fold increase, respectively, for the colon tumors.

In contrast, the amplification of the closest known framework markers (from Table 10) and epicenter markers (Table 10.2) does not occur to a greater extent than that of DNA58125. This strongly suggests that DNA58125 is the gene responsible for the amplification of the particular region on chromosome 16. Because amplification of DNA58125 occurs in various tumor tissues, especially colon tumors, it is highly probable to play a significant role in tumor formation or growth, in particular, colon tumor formation and growth. As a result, antagonist (e.g., antibodies) directed against the protein encoded by DNA58125 would be expected to have utility in cancer therapy.

### EXAMPLE 3

#### In situ Hybridization

*In situ* hybridization is a powerful and versatile technique for the detection and localization of nucleic acid sequences within cell or tissue preparations. It may be useful, for example, to identify sites of gene expression, analyze the tissue distribution of transcription, identify and localize viral infection, follow changes in specific mRNA synthesis and aid in chromosome mapping.

*In situ* hybridization was performed following an optimized version of the protocol by Lu and Gillett, Cell Vision 1: 169-176 (1994), using PCR-generated  $^{32}\text{P}$ -labeled riboprobes. Briefly, formalin-fixed, paraffin-embedded human tissues were sectioned, deparaffinized, deproteinized in proteinase K (20 g/ml) for 15 minutes at 37°C, and further processed for *in situ* hybridization as described by Lu and Gillett, *supra*. A [ $^{32}\text{P}$ ] UTP-labeled antisense riboprobe was generated from a PCR product

and hybridized at 55°C overnight. The slides were dipped in Kodak NTB2 nuclear track emulsion and exposed for 4 weeks.

#### <sup>33</sup>P-Riboprobe synthesis

- 6.0 µl (125 mCi) of <sup>33</sup>P-UTP (Amersham BF 1002, SA<2000 Ci/mmol) were speed vac  
 5 dried. To each tube containing dried <sup>33</sup>P-UTP, the following ingredients were added:
- 2.0 µl 5x transcription buffer
  - 1.0 µl DTT (100 mM)
  - 2.0 µl NTP mix (2.5 mM : 10 µl; each of 10 mM GTP, CTP & ATP + 10 µl H<sub>2</sub>O)
  - 1.0 µl UTP (50 µM)
  - 10 1.0 µl Rnasin
  - 1.0 µl DNA template (1 µg)
  - 1.0 µl H<sub>2</sub>O
  - 1.0 µl RNA polymerase (for PCR products T3 = AS, T7 = S, usually)

- The tubes were incubated at 37°C for one hour. 1.0 µl RQ1 DNase were added,  
 15 followed by incubation at 37°C for 15 minutes. 90 µl TE (10 mM Tris pH 7.6/1mM EDTA pH 8.0) were added, and the mixture was pipetted onto DE81 paper. The remaining solution was loaded in a Microcon-50 ultrafiltration unit, and spun using program 10 (6 minutes). The filtration unit was inverted over a second tube and spun using program 2 (3 minutes). After the final recovery spin, 100 µl TE were added. 1 µl of the final product was pipetted on DE81 paper and counted in 6 ml of Biofluor II.

- 20 The probe was run on a TBE/urea gel. 1-3 µl of the probe or 5 µl of RNA Mrk III were added to 3 µl of loading buffer. After heating on a 95°C heat block for three minutes, the gel was immediately placed on ice. The wells of gel were flushed, the sample loaded, and run at 180-250 volts for 45 minutes. The gel was wrapped in saran wrap and exposed to XAR film with an intensifying screen in -70°C freezer one hour to overnight.

#### 25 <sup>33</sup>P-Hybridization

- Pretreatment of frozen sections* The slides were removed from the freezer, placed on aluminium trays and thawed at room temperature for 5 minutes. The trays were placed in 55°C incubator for five minutes to reduce condensation. The slides were fixed for 10 minutes in 4% paraformaldehyde on ice in the fume hood, and washed in 0.5 x SSC for 5 minutes, at room temperature (25 ml 20 x SSC + 975  
 30 ml SQ H<sub>2</sub>O). After deproteinization in 0.5 µg/ml proteinase K for 10 minutes at 37°C (12.5 µl of 10 mg/ml stock in 250 ml prewarmed RNase-free RNase buffer), the sections were washed in 0.5 x SSC for 10 minutes at room temperature. The sections were dehydrated in 70%, 95%, 100% ethanol, 2 minutes each.

- Pretreatment of paraffin-embedded sections* The slides were deparaffinized, placed in SQ H<sub>2</sub>O, and rinsed twice in 2 x SSC at room temperature, for 5 minutes each time. The sections were  
 35 deproteinized in 20 µg/ml proteinase K (500 µl of 10 mg/ml in 250 ml RNase-free RNase buffer; 37°C, 15 minutes) - human embryo, or 8 x proteinase K (100 µl in 250 ml RNase buffer, 37°C, 30 minutes) - formalin tissues. Subsequent rinsing in 0.5 x SSC and dehydration were performed as described above.

- Prehybridization* The slides were laid out in plastic box lined with Box buffer (4 x SSC, 50% formamide) - saturated filter paper. The tissue was covered with 50 µl of hybridization buffer (3.75g  
 40 Dextran Sulfate + 6 ml SQ H<sub>2</sub>O), vortexed and heated in the microwave for 2 minutes with the cap

loosened. After cooling on ice, 18.75 ml formamide, 3.75 ml 20 x SSC and 9 ml SQ H<sub>2</sub>O were added, the tissue was vortexed well, and incubated at 42°C for 1-4 hours.

- Hybridization* 1.0 x 10<sup>6</sup> cpm probe and 1.0 µl tRNA (50 mg/ml stock) per slide were heated at 95°C for 3 minutes. The slides were cooled on ice, and 48 µl hybridization buffer were added per slide. After vortexing, 50 µl <sup>32</sup>P mix were added to 50 µl prehybridization on slide. The slides were incubated overnight at 55°C.

- Washes* Washing was done 2x10 minutes with 2xSSC, EDTA at room temperature (400 ml 20 x SSC + 16 ml 0.25M EDTA, V<sub>F</sub>=4L), followed by RNaseA treatment at 37°C for 30 minutes (500 µl of 10 mg/ml in 250 ml Rnase buffer = 20 µg/ml). The slides were washed 2x10 minutes with 2 x SSC, EDTA at room temperature. The stringency wash conditions were as follows: 2 hours at 55°C, 0.1 x SSC, EDTA (20 ml 20 x SSC + 16 ml EDTA, V<sub>F</sub>=4L).

#### DNA38113 (Prolactin Receptor Homolog)

- DNA38113-p1: GGA TTC TAA TAC GAC TCA CTA TAG GGC CCC CCT GAG CTC TCC CGT  
GTA  
15 DNA38113-p2: CTA TGA AAT TAA CCC TCA CTA AAG GGA AGG CTC GCC ACT GGT CGT  
AGA

- High expression was observed in developing mouse and human fetal lung (Fig. 37), while normal adult lung, including bronchial epithelium was negative. Expression was also seen in human fetal trachea, including with high probability, smooth muscle cells (Fig. 38). Expression was also observed in non-trophoblastic cells in the human placenta (Fig. 39). These data are consistent with a potential role in bronchial development.

#### DNA44804 (ALS Homolog)

- 608.p1: GGA TTC TAA TAC GAC TCA CTA TAG GGC TGC CCG CAA CCC CTT CAA CTG  
608.p2: CTA TGA AAT TAA CCC TCA CTA AAG GGA CCG CAG CTG GGT GAC CGT GTA

- 25 Low to moderate expression at sites of bone formation in fetal tissues and in the malignant cells of an osteosarcoma. Expression was also observed at low level in the placenta and umbilical cord.

#### DNA52722 (TNF Homolog)

- dna52722p1: GGA TTC TAA TAC GAC TCA CTA TAG GGC CGC CCC GCC ACC TCC T  
dna52722p2: CTA TGA AAT TAA CCC TCA CTA AAG GGA CTC GAG ACA CCA CCT GAC  
30 CCA  
52722.p3: GGA TTC TAA TAC GAC TCA CTA TAG GGC CCA AGG AAG GCA GGA GAC  
TCT  
52722.p4: CTA TGA AAT TAA CCC TCA CTA AAG GGA CTA GGG GGT GGG AAT GAA  
AAG

High expression levels were observed in many tissues, including placenta, osteoblasts, injured renal tubules, injured liver colorectal liver metastasis and gall bladder. Tested sample had acetaminophen induced liver injury and hepatic cirrhosis.

DNA48227 (Reductase Homolog)

- 5 607.p1: GGA TTC TAA TAC GAC TCA CTA TAG GGC CCA ACA GCG GCA TCG GAA AGA  
607.p2: CTA TGA AAT TAA CCC TCA CTA AAG GGA GGA GCA CCA GCC AAG CCA ATG

Elevated expression was observed in the mucosa of the chimp stomach.

EXAMPLE 4

Use of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715,

- 10 PRO1017, PRO1112, PRO509, PRO853 or PRO882 as a hybridization probe

The following method describes use of a nucleotide sequence encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide as a hybridization probe.

- 15 DNA comprising the coding sequence of full-length or mature PRO201 (Fig.1, SEQ ID NO: 1), PRO292 (Fig. 3, SEQ ID NO: 3), PRO327 (Fig. 5, SEQ ID NO: 5), PRO1265 (Fig. 7, SEQ ID NO: 7), PRO344 (Fig. 9, SEQ ID NO: 9), PRO343 (Fig. 11, SEQ ID NO: 11), PRO347 (Fig. 13, SEQ ID NO: 13), PRO357 (Fig. 15, SEQ ID NO: 15), PRO715 (Fig. 17, SEQ ID NO: 17), PRO1017 (Fig. 19, SEQ ID NO: 19), PRO1112 (Fig. 21, SEQ ID NO: 21), PRO509 (Fig. 23, SEQ ID NO: 23), PRO853 (Fig. 25, SEQ ID NO: 25) or PRO882 (Fig. 27, SEQ ID NO: 27) is employed as a probe to screen for  
20 homologous DNAs (such as those encoding naturally-occurring variants of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in human tissue cDNA libraries or human tissue genomic libraries.

- Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO201, PRO292, PRO327,  
25 PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

- 30 DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can then be identified using standard techniques known in the art.

EXAMPLE 5

- 35 Expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715,  
PRO1017, PRO1112, PRO509, PRO853 or PRO882 in *E. coli*

This example illustrates preparation of an unglycosylated form of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 by recombinant expression in *E. coli*.

The DNA sequence encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 (SEQ ID NOs: 1; 3; 5; 7; 9; 11; 13; 15; 17; 19; 21; 23; 25; 27, respectively) is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

#### EXAMPLE 6

##### Expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO201, PRO292, PRO327, PRO1265, PRO344,

PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 DNA using

ligation methods such as described in Sambrook et al., *supra*. The resulting vector is called pRK5-PRO201, pRK5-PRO292, pRK5-PRO327, pRK5-PRO1265, pRK5-PRO344, pRK5-PRO343, pRK5-PRO347, pRK5-PRO357, pRK5-PRO715, pRK-1017, pRK5-PRO1112, pRK5-PRO509, pRK5-PRO853, pRK5-PRO882.

- 5 In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10  $\mu$ g pRK5-PRO201, pRK5-PRO292, pRK5-PRO327, pRK5-PRO1265, pRK5-PRO344, pRK5-PRO343, pRK5-PRO347, pRK5-PRO357, pRK5-PRO715, pRK-1017, pRK5-PRO1112, pRK5-PRO509, pRK5-PRO853, pRK5-PRO882 DNA is mixed with about 1  $\mu$ g DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500  $\mu$ l of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M  $\text{CaCl}_2$ . To this mixture is added, dropwise, 500  $\mu$ l of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM  $\text{NaPO}_4$ , and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

- Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200  $\mu$ Ci/ml  $^{35}\text{S}$ -cysteine and 200  $\mu$ Ci/ml  $^{35}\text{S}$ -methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, or EBAF-2 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

- 25 In an alternative technique, PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 DNA may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac et al., *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700  $\mu$ g pRK5-PRO201, pRK5-PRO292, pRK5-PRO327, pRK5-PRO1265, pRK5-PRO344, pRK5-PRO343, pRK5-PRO347, pRK5-PRO357, pRK5-PRO715, pRK-1017, pRK5-PRO1112, pRK5-PRO509, pRK5-PRO853, pRK5-PRO882 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5  $\mu$ g/ml bovine insulin and 0.1  $\mu$ g/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

- In another embodiment, PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can be expressed in

CHO cells. The pRK5-PRO201, pRK5-PRO292, pRK5-PRO327, pRK5-PRO1265, pRK5-PRO344, pRK5-PRO343, pRK5-PRO347, pRK5-PRO357, pRK5-PRO715, pRK-1017, pRK5-PRO1112, pRK5-PRO509, pRK5-PRO853, pRK5-PRO882 vector can be transfected into CHO cells using known reagents such as  $\text{CaPO}_4$  or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as  $^{35}\text{S}$ -methionine. After determining the presence of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can then be concentrated and purified by any selected method.

Epitope-tagged PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 may also be expressed in host CHO cells. The PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-His tag into a Baculovirus expression vector. The poly-His tagged PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

PRO292 (UNQ266) and PRO509 (UNQ329) were expressed in CHO cells by a transient expression procedure. In addition, PRO1017 (UNQ500) and PRO509 (UNQ329) were stably expressed in CHO cells.

Stable expression in CHO cells was performed using the following procedure. The proteins were expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins were fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs were subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., *Nucl. Acids Res.* 24: 9 (1774-1779) (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA were introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Quiagen), Dospacer® or Eugene® (Boehringer Mannheim). The cells were grown and described in Lucas *et al.*, supra. Approximately  $3 \times 10^7$  cells are frozen in an ampule for further growth and production as described

5 below.

The ampules containing the plasmid DNA were thawed by placement into water bath and mixed by vortexing. The contents were pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were resuspended in 10 mL of selective media (0.2  $\mu$ m filtered PS20 with 5% 0.2  $\mu$ m diafiltered fetal bovine serum). The cells were then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells were transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, a 250 mL, 500 mL and 2000 mL spinners were seeded with  $3 \times 10^5$  cells/mL. The cell media was exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in US Patent No. 5,122,469, issued June 16, 1992 was actually used. 3L production spinner is seeded at  $1.2 \times 10^6$  cells/mL. On day 0, the cell number pH were determined. On day 1, the spinner was sampled and sparging with filtered air was commenced. On day 2, the spinner was sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion). Throughout the production, pH was adjusted as necessary to keep at around 7.2. After 10 days, or until viability dropped below 70%, the cell culture was harvested by centrifugation and filtering through a 0.22  $\mu$ m filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media was pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of were purified from the conditioned media as follows. The conditioned medium was pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275  $\mu$ L of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity was assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.



PRO292 (UNQ266), PRO327 (UNQ288), PRO344 (UNQ303), PRO347 (UNQ306), PRO357 (UNQ314), PRO853 (UNQ419) were also produced by transient expression in COS cells.

#### EXAMPLE 7

5 Expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in Yeast

The following method describes recombinant expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in yeast.

10 First, yeast expression vectors are constructed for intracellular production or secretion of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 from the ADH2/GAPDH promoter. DNA encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 and the promoter is inserted into suitable restriction enzyme sites in the  
15 selected plasmid to direct intracellular expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882. For secretion, DNA encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

25 Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

30 Recombinant PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 may further be purified using selected column chromatography resins.

## EXAMPLE 8

Expression of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 in Baculovirus-Infected Insect Cells

The following method describes recombinant expression in Baculovirus-infected insect  
 5 cells.

The sequence coding for PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including  
 10 plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 or the desired portion of the coding sequence of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 [such as the sequence encoding the extracellular  
 15 domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular] is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and  
 20 BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

Expressed poly-His tagged PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared  
 30 by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 µm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at  
 35 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot  
 40 with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged

PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, respectively are pooled and dialyzed against loading buffer.

- Alternatively, purification of the IgG tagged (or Fc tagged) PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

- PRO327 (UNQ288), PRO344 (UNQ303), PRO509 (UNQ329) were expressed in baculovirus infected Sf9 insect cells. While the expression was actually performed in a 0.5-2 L scale, it can be readily scaled up for larger (e.g. 8 L) preparations. The proteins were expressed as an IgG construct (immunoadhesin), in which the protein extracellular region was fused to an IgG1 constant region sequence containing the hinge, CH2 and CH3 domains and/or in poly-His tagged forms.

- Following PCR amplification, the respective coding sequences were subcloned into a baculovirus expression vector (pb.PH.IgG for IgG fusions and pb.PH.His.c for poly-His tagged proteins), and the vector and Baculogold® baculovirus DNA (Pharmingen) were co-transfected into 105 *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711), using Lipofectin (Gibco BRL). pb.PH.IgG and pb.PH.His are modifications of the commercially available baculovirus expression vector pVL1393 (Pharmingen), with modified polylinker regions to include the His or Fc tag sequences. The cells were grown in Hink's TNM-FH medium supplemented with 10% FBS (Hyclone). Cells were incubated for 5 days at 28°C. The supernatant was harvested and subsequently used for the first viral amplification by infecting Sf9 cells in Hink's TNM-FH medium supplemented with 10% FBS at an approximate multiplicity of infection (MOI) of 10. Cells were incubated for 3 days at 28°C. The supernatant was harvested and the expression of the constructs in the baculovirus expression vector was determined by batch binding of 1 ml of supernatant to 25 mL of Ni-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

The first viral amplification supernatant was used to infect a spinner culture (500 ml) of Sf9 cells grown in ESF-921 medium (Expression Systems LLC) at an approximate MOI of 0.1. Cells were incubated for 3 days at 28°C. The supernatant was harvested and filtered. Batch binding and SDS-PAGE analysis was repeated, as necessary, until expression of the spinner culture was confirmed.

- The conditioned medium from the transfected cells (0.5 to 3 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein construct were purified using a Ni-NTA column (Qiagen). Before purification, imidazole was added to the conditioned media to a concentration of 5 mM. The conditioned media were pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins were purified from the conditioned media as follows. The conditioned media were pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5.

- 5 The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 mL of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the proteins was verified by SDS polyacrylamide gel (PEG) electrophoresis and N-terminal amino acid sequencing by Edman degradation.

- 10 Additionally, PRO1265 (UNQ636), PRO344 (UNQ303), PRO882 (UNQ448) were expressed by a modified baculovirus procedure in high-5 cells.

- The DNA encoding the desired sequence was amplified with suitable systems, such as Pfu (Stratagene), or fused upstream (5'-of) of an epitope tag contained with a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pIE1-1 (Novagen). The pIE1-1 and pIE1-2 vectors are designed for constitutive expression of recombinant proteins from the baculovirus ie1 promoter in stably-transformed insect cells (1). The plasmids differ only in the orientation of the multiple cloning sites and contain all promoter sequences known to be important for ie1-mediated gene expression in uninfected insect cells as well as the hr5 enhancer element. pIE1-1 and pIE1-2 include the ie translation initiation site and can be used to produce fusion proteins. Briefly, the desired sequence or the desired portion of the sequence (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product was then digested with those selected restriction enzymes and subcloned into the expression vector. For example, derivatives of pIE1-1 can include the Fc region of human IgG (pb.PH.IgG) or an 8 histidine (pb.PH.His) tag downstream (3'-of) the desired sequence. Preferably, the vector construct is sequenced for confirmation.
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- Hi5 cells are grown to a confluency of 50% under the conditions of, 27°C, no CO<sub>2</sub>, NO pen/strep. For each 150 mm plate, 30 ug of pIE based vector containing the sequence was mixed with 1 ml Ex-Cell medium (Media: Ex-Cell 401 + 1/100 L-Glu JRH Biosciences #14401-78P (note: this media is light sensitive)), and in a separate tube, 100 ul of CellFectin (CellFECTIN (GibcoBRL #10362-010) (vortexed to mix)) was mixed with 1 ml of Ex-Cell medium. The two solutions were combined and allowed to incubate at room temperature for 15 minutes. 8 ml of Ex-Cell media was added to the 2ml of DNA/CellFECTIN mix and this is layered on Hi5 cells that have been washed once with Ex-Cell media. The plate is then incubated in darkness for 1 hour at room temperature. The DNA/CellFECTIN mix is then aspirated, and the cells are washed once with Ex-Cell to remove excess CellFECTIN. 30 ml of fresh Ex-Cell media was added and the cells are incubated for 3 days at 28C. The supernatant was harvested and the expression of the sequence in the baculovirus expression vector was determined by batch binding of 1 ml of supernatant to 25 mL of Ni-NTA beads (QIAGEN) for histidine tagged proteins or Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.
- 30
- 35
- 40

The conditioned media from the transfected cells (0.5 to 3 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein comprising the sequence is purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media was pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 48°C. After loading, the column was washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein was then subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc containing) constructs of proteins were purified from the conditioned media as follows. The conditioned media was pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column was washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein was immediately neutralized by collecting 1 ml fractions into tubes containing 275 mL of 1 M Tris buffer, pH 9. The highly purified protein was subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity of the sequence was assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation and other analytical procedures as desired or necessary.

#### EXAMPLE 9

##### Preparation of Antibodies that Bind PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, fusion proteins containing PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882, and cells expressing recombinant PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected

adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti- PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 antibodies.

- 5                   After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available  
10 from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

- The hybridoma cells will be screened in an ELISA for reactivity against PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112,  
15 PRO509, PRO853 or PRO882. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 is within the skill in the art.

- The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti- PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343,  
20 PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

25                   \* \* \* \*

#### Deposit of Material

                  The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
30	DNA30676-1223	209567	12/23/97
	DNA38113-1230	209530	12/10/97
	DNA60764-1533	203452	11/10/98
	DNA40592-1242	209492	11/21/97
	DNA43318-1217	209482	11/21/97
35	DNA44176-1244	209532	12/10/97
	DNA44804-1248	209527	12/10/97
	DNA52722-1229	209570	1/7/98
	DNA56112-1379	209883	5/20/98
	DNA57702-1476	209951	6/9/98
40	DNA48227-1350	209812	4/28/98

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

## Claims:

1. An isolated antibody binding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.
2. The antibody of claim 1 which induces death of a cell overexpressing a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.
3. The antibody of claim 2 wherein said cell is a cancer cell.
4. The antibody of claim 1 which is a monoclonal antibody.
5. The antibody of claim 4, which has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.
6. The antibody of claim 5 which is labeled.
7. The antibody of claim 6 which is immobilized on a solid support.
8. The antibody of claim 1 which is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.
9. A composition comprising an antibody of claim 1 in admixture with a pharmaceutically acceptable carrier.
10. The composition of claim 9 comprising growth inhibitory amount of said antibody.
11. The composition of claim 10 further comprising a second antibody or a cytotoxic or chemotherapeutic agent.
12. A method for determining the presence of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide comprising exposing a cell suspected of containing the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide to an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and determining binding of said antibody to said cell.
13. A method of diagnosing tumor in a mammal, comprising detecting the level of expression of a gene encoding a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher expression level in the test sample indicates the presence of tumor in the mammal from which the test tissue cells were obtained.
14. A method of diagnosing tumor in a mammal, comprising (a) contacting an PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347,



- PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide in the test sample.
15. The method of claim 14 wherein said test sample is obtained from an individual suspected to have neoplastic cell growth or proliferation.
- 5 16. A cancer diagnostic kit, comprising an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody and a carrier in suitable packaging.
- 10 17. The kit of claim 16 further comprising instructions for using said antibody to detect the PRO187, PRO533, PRO214, PRO240, PRO211, PRO230, PRO261, PRO246, or EBAF-2 polypeptide.
18. A method for inhibiting the growth of tumor cells comprising exposing a cell which overexpresses a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide to an effective amount of an agent inhibiting the expression and/or activity of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.
- 15 19. The method of claim 18 wherein said agent is an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody.
- 20 20. The method of claim 19 wherein said tumor cells are further exposed to radiation treatment or a cytotoxic or chemotherapeutic agent.
21. An article of manufacture, comprising:
- 25 a container;
- a label on the container; and
- a composition comprising an active agent contained within the container; wherein the composition is effective for inhibiting the growth of tumor cells, the label on the container indicates that the composition can be used for treating conditions characterized by overexpression of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, and the active agent in the composition is an agent inhibiting the expression and/or activity of the PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide.
- 30 22. The article of manufacture of claim 21 wherein said active agent is an anti-PRO201, anti-PRO292, anti-PRO327, anti-PRO1265, anti-PRO344, anti-PRO343, anti-PRO347, anti-PRO357, anti-PRO715, anti-PRO1017, anti-PRO1112, anti-PRO509, anti-PRO853 or anti-PRO882 antibody.
- 35 23. A method for identifying a compound capable of inhibiting the expression or activity of a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide, comprising contacting a
- 40

candidate compound with a PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide under conditions and for a time sufficient to allow these two components to interact.

24. The method of claim 23 wherein said candidate compound or said PRO201, PRO292, PRO327, PRO1265, PRO344, PRO343, PRO347, PRO357, PRO715, PRO1017, PRO1112, PRO509, PRO853 or PRO882 polypeptide is immobilized on a solid support.
25. The method of claim 24 wherein the non-immobilized component carries a detectable label..

1 / 39

SEQ ID NO:1

CGGGGGTGAC	AGCAGCCCCG	AGCCGCGGAG	CCTCAGCTTC	CGCCTGGACC
CAGCCTCGTG	GGAGCCCCGC	GGGTCTTGCC	CAGATGTGGA	AGACTGAGGC
CTGTTGAAAG	TGCAGAGCTC	AGCCCTGGCA	CCCTCTGTTT	CCAAGAGCTC
CATGCAGGTG	CCACAGGATG	GAGAAGACCT	TGCTGGCCAA	CCCTGGTACC
ACGGCCTCCT	GTCCCGCCAG	AAGGCTGAAG	CTCTTCTTCA	GCAAGATGGC
GACTTCCTGG	TTCGCGCCTC	TGGGTCCCCT	GGGGGCAACC	CCGTGATCTC
CTGCCGCTGG	CGGGGCTCAG	CCCTCCATTT	TGAGGTGTTT	CGTGTGGCCC
TGCGTCCCCG	GCCAGGCCGA	CCCACAGCCC	TCTTTCAACT	GGAGGATGAG
CAATTCCCCA	GCATACCGGC	TCTGGTTCAC	AGTTATATGA	CAGGCAGGCG
CCCCTGTGCC	CAGGCCACAG	GGGCTGTGGT	CTCCAGGCCCT	GTGACTTGGC
AGGGGCCTCT	GCGACGCAGC	TTTAGCGAGG	ACACCCTGAT	GGATGGCCCCA
GCTCGGATAG	AGCCTCTCAG	GGCAAGGAAG	TGGAGCAACA	GTCAGCCTGC
AGATTTGGCA	CATATGGGGC	GGTCAAGAGA	AGACCCCGCT	GGGATGGAAG
CCTCCACCAT	GCCCATATCT	GCCTTGCCCC	GAACGAGCAG	TGACCCGGTG
TTGCTGAAGG	CCCCTGCTCC	CCTGGGAAC	GTTGCCGACA	GTCTCAGGGC
CTCCGATGGG	CAGCTTCAAG	CCAAGGCACC	AACGAAGCCC	CCCCGGACAC
CCTCCTTCGA	ACTGCCTGAT	GCCTCTGAAC	GTCCCCCGAC	GTACTGCGAG
CTGGTGCCCC	GAGTGCCCG	TGTCCAGGGA	ACATCCCCGA	GCCAAAGCTG
CCCAGAGCCA	GAGGCCCCAT	GGTGGGAGGC	CGAGGAGGAT	GAGGAGGAAG
AGAATAGATG	TTTTACAAGA	CCACAGGCTG	AGATCTCTTT	CTGCCCCCAT
GATGCCCCCT	CCTGCCTGCT	GGGCCCCCAG	AATCGGCCCC	TGGAACCCCCA
AGTCCTGCAT	ACCCTCCGTG	GCCTGTTTCT	GGAGCACCAT	CCTGGGAGCA
CCGCCCTTCA	CCTGCTATTG	GTAGACTGCC	AGGCCACAGG	CCTCCTGGGA
GTGACCAGAG	ATCAGCGGGG	CAACATGGGA	GTCTCATCTG	GCCTGGAGCT
GCTCACTCTT	CCCCATGGAC	ACCACTTGAG	GTTGGAAC	CTGGAGAGGC
ATCAGACACT	GGCGCTGGCC	GGGGCGCTGG	CGGTGCTGGG	CTGCTCGGGG
CCGCTGGAGG	AGCGCGCAGC	CGCACTGAGG	GGACTGGTAG	AGCTGGCGCT
GGCGCTGCGG	CCAGGGGCGG	CGGGGGACCT	GCCCCGGGCTG	GCTGCAGTCA
TGGGCGCCCT	GCTCATGCCC	CAGGTGTCCC	GGTTGGAGCA	CACGTGGCGC
CAGCTCCGAA	GGAGCCACAC	GGAGGCTGCG	CTGGCCTTTG	AGCAGGAGCT
GAAGCCGCTG	ATGCGGGCTC	TGGATGAGGG	CGCTGGACCC	TGCGACCCCCG
GCGAGGTGGC	GCTGCCGCAC	GTGGCACCCA	TGGTTCGCCT	ACTGGAGGGC
GAGGAAGTCG	CGGGGCGGCT	GGACGAGAGC	TGTGAGCGGC	TGTTGCGCAC
CCTGCACGGG	GCGCGTCACA	TGGTCCGGGA	CGCACCCAAA	TTCCGCAAGG
TGGCAGCCCA	GCGCCTGCGA	GGATTCCGGC	CTAACCCGGA	GCTGAGGGAG
GCCCTGACCA	CCGGCTTCGT	GCGGAGGCTG	CTCTGGGGTA	GCCGGGGCGC
GGGAGCTCCG	CGCGCTGAAC	GCTTTGAGAA	GTTCCAGCGC	GTCCTCGGCG
TCCTGTGCGA	GCGCCTGGAG	CCTGACCGCT	GAGAGCGCAG	ACACCCTTCT
TCACACCCGG	GACCCCCAGG	TTTTTGCGAA	CCCCAGAAGA	GACCAAAGGA
GTCGTCCCAG	GCTCCTCGCG	CCTCAGGTGG	AATCCTGCCC	TGTGCCTCAC
AGAAGAGGTG	GGGACCGCAG	TCAGGGTCAC	CTGGACCATG	GTGAACATGT
GACCTGCAGA	TCTGGCATCA	GAGGCCAGAG	TTCAAATGTG	ACTCCACCTC
TTAAAAGCCG	TGATTTCTAG	CAGTTGACTT	CACCTCTGTG	TCGGCCTTTA
ACAAAATCAT	AGCCATACAG	CAGCTCAGGC	CTGTAATCTC	AGCACTTTGG
GAGGCCGAGG	CGGAAGGAAG	GCTTGAGGCC	AGGAGTTCAA	GACCAGCCAG
GGCAACATGG	TGAGACCTCA	TCTCTACAAA	AACTGAAAAA	TAAAAAACTT
TTAAAAAATG	TAAAAAATAA	AAAAAAAGGG	CGGCCGCGAC	TCTAGAGTCG
ACCTGCAGAA	GCTTGGCCGC	CATGGCCCAA	CTTGTTTATT	GCAGCTTATA
ATGTTACAA	ATA			

FIG. 1

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SEQ ID NO:2

MQVPQDGEDLAGQPWYHGLLSRQKAEALLQQDGDFLVRASGSRGGNPVISCRWRGSAHF  
EVRVALRPRPGRPTALFQLEDEQFPSPALVHSYMTGRRPLSQATGAVVSRPVTWQGPL  
RRSFSEDTLMDGPARIEPLRAKWSNSQPADLAHMGRSREDPAGMEASTMPI SALTPTSS  
DPVLLKAPAPLGTVADSLRASDGQLQAKAPT KPPRTPSFELPDASERPPTYCELVPRVPS  
VQGTSPSQSCPEPEAPWWEAEDEEEENRCFTRPQAEISFCPHDAPSCLLGPQNRPLEPQ  
VLHTLRGLFLEHHPGSTALHLLVDCQATGLLGVTDRQGRNMGVSSGLELLTLPHGHHLR  
LELLERHQTLALAGALAVLGCSPLEERAAALRGLVELALALRPGAAGDLPGLAAVMGAL  
LMPQVSRLEHTWRQLRRSHTEAALAFEQELKPLMRALDEGAGPCDPGEVALPHVAPMVR  
LEGEVAGPLDESCERLLRTLHGARMVRDAPKFRKVAQQLRGFRPNPELREALTTGFV  
RRLWGSRGAGAPRAERFEKFQVRLGVLSQRLPEPDR

FIG. 2

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SEQ ID NO:3

ATGCAGCCCTCCAGCCTTCTGCCGCTCGCCCTCTGCCTGCTGGCTGCACCCGCTCCGCG  
CTCGTCAGGATCCCGCTGCACAAGTTCACGTCCATCCGCCGGACCATGTCGGAGGTGGG  
GGCTCTGTGGAGGACCTGATTGCCAAAGGCCCCGTCTCAAAGTACTCCCAGGCGGTGCCA  
GCCGTGACCGAGGGGCCCCATTCCCGAGGTGCTCAAGAACTACATGGACGCCCAGTACTAC  
GGGGAGATTGGCATCGGGACGCCCCCCCAGTGCTTCACAGTCGTCTTCGACACGGGCTCC  
TCCAACCTGTGGGTCCCCCTCCATCCACTGCAAAGTCTGGACATCGCTTGCTGGATCCAC  
CACAAGTACAACAGCGACAAGTCCAGCACCTACGTGAAGAATGGTACCTCGTTTGACATC  
CACTATGGCTCGGGCAGCCTCTCCGGGTACCTGAGCCAGGACACTGTGTGGTGCCCTGC  
CAGTCAGCGTCGTGAGCCTCTGCCCTGGGCGGTGTCAAAGTGGAGAGGCAGGTCTTTGGG  
GAGGCCACCAAGCAGCCAGGCATCACCTTCATCGCAGCCAAGTTCGATGGCATCCTGGGC  
ATGGCCTACCCCCGCATCTCCGTCAACAACGTGCTGCCCCGTCTTCGACAACCTGATGCAG  
CAGAAGCTGGTGGACCAGAACATCTTCTCCTTCTACCTGAGCAGGGACCCAGATGCGCAG  
CCTGGGGGTGAGCTGATGCTGGGTGGCACAGACTCCAAGTATTACAAGGGTTCTCTGTCC  
TACCTGAATGTCACCCGCAAGGCCTACTGGCAGGTCCACCTGGACCAGGTGGAGGTGGCC  
AGCGGGCTGACCCTGTGCAAGGAGGGCTGTGAGGCCATTGTGGACACAGGCACTTCCCTC  
ATGGTGGGCCCCGGTGGATGAGGTGCGCGAGCTGCAGAAGGCCATCGGGGCCGTGCCGCTG  
ATTCAGGGCGAGTACATGATCCCCTGTGAGAAGGTGTCCACCCTGCCCGCGATCACACTG  
AAGCTGGGAGGCAAAGGCTACAAGCTGTCCCCAGAGGACTACACGCTCAAGGTGTGCGAG  
GCCGGGAAGACCTCTGCCTGAGCGGCTTCATGGGCATGGACATCCCGCCACCCAGCGGG  
CCACTCTGGATCCTGGGCGACGTCTTCATCGGCCGCTACTACACTGTGTTTGACCGTGAC  
AACAACAGGGTGGGCTTCGCCGAGGCTGCCCCGCTCTAGTTCCCAAGGCGTCCGCGCGCC  
AGCACAGAAACAGAGGAGAGTCCCAGAGCAGGAGGCCCCCT

FIG. 3

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SEQ ID NO:4

MQPSSLLPLALCLLAAPASALVRIPLHKFTSIRRTMSEVGGSVEDLIAKGPVSKYSQAV  
PAVTEGPIPEVLKNYMDAQYYGEIGIGTPPQCFTVVFDTGSSNLWVPSIHCKLLDIACW  
IHHKYNSDKSSTYVKNGTSFDIHYGSGSLSGYLSQDTVSVPCQSASSASALGGVKVERQ  
VFGEATKQPGITFIAAKFDGILGMAYPRISVNNVLPVFDNLMQQKLVDQNI FSFYLSRD  
PDAQPGGELMLGGTDSKYYKGSLSYLNVTRKAYWQVHLDQVEVASGLTLCKEGCEAIVD  
TGTSLMVGPVDEVRELQKAIGAVPLIQGEYMIPCEKVSTLPAITLKLGGKGYKLSPEDY  
TLKVSQAGKTLCLSGFMGMDIPPPSGPLWILGDVFIGRYYTVFDRDNNRVGFAEAAARLO

**FIG. 4**

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SEQ ID NO:5

CGCCCAGCGACGTGCGGGCGGCCTGGCCCCGCGCCCTCCCGCGCCCGGCCTGCGTCCCGCG  
CCCTGCGCCACCGCCGCGGAGCCGCGAGCCCGCGCGCGCCCCCGGCAGCGCCGGCCCC  
><Met {trans=1-s, dir=f, res=1}>  
ATGCCCCGCGGCGCGGGGCCCCGCGCCCAATCCGCGCGGCGGCGCGCGCGTGTGCTG  
CCCCTGCTGCTGCTGCTCTGCGTCCTCGGGGCGCCGCGAGCCGGATCAGGAGCCACACA  
GCTGTGATCAGTCCCCAGGATCCCACGCTTCTCATCGGCTCCTCCCTGCTGGCCACCTGC  
TCAGTGACGGAGACCCACCAGGAGCCACCGCCGAGGGCCTCTACTGGACCCTCAACGGG  
CGCCGCCTGCCCCCTGAGCTCTCCCGTGTAACAACGCCTCCACCTTGGCTCTGGCCCTG  
GCCAACCTCAATGGGTCCAGGCAGCGGTGCGGGGACAACCTCGTGTGCCACGCCCCGTGAC  
GGCAGCATCCTGGCTGGCTCCTGCCTCTATGTTGGCCTGCCCCCAGAGAAACCCGTCAAC  
ATCAGCTGCTGGTCCAAGAACATGAAGGACTTGACCTGCCGCTGGACGCCAGGGGCCCCAC  
GGGGAGACCTTCCTCCACACCAACTACTCCCTCAAGTACAAGCTTAGGTGGTATGGCCAG  
GACAACACATGTGAGGAGTACCACACAGTGGGGCCCCACTCCTGCCACATCCCCAAGGAC  
CTGGCTCTCTTTACGCCCTATGAGATCTGGGTGGAGGCCACCAACCGCCTGGGCTCTGCC  
CGCTCCGATGTACTCACGCTGGATATCCTGGATGTGGTGACCACGGACCCCCCGCCGAC  
GTGCACGTGAGCCGCGTCGGGGGCTGGAGGACCAGCTGAGCGTGCGCTGGGTGTGCCA  
CCCGCCCTCAAGGATTTCTCTTTCAAGCCAAATACCAGATCCGCTACCGAGTGGAGGAC  
AGTGTGGAAGTGGTGGACGATGTGAGCAACCAGACCTCCTGCCGCCTGGCCGGC  
CTGAAACCCGGCACCGTGTACTTCGTGCAAGTGCGCTGCAACCCCTTTGGCATCTATGGC  
TCCAAGAAAGCCGGGATCTGGAGTGAGTGAGCCACCCACAGCCGCCTCCACTCCCCGC  
AGTGAGCGCCCGGGCCCGGGCGGGGCGTGCGAACCGCGGGGCGGAGAGCCGAGCTCG  
GGGCCGTTGCGGCGCGAGCTCAAGCAGTTCCTGGGCTGG

CTCAAGAAGCACGCGTACTGCT

CCAACCTCAGCTTCGCCTCTACGACCAGTGGCGAGCCTGGAT

GCAGAAGTCGCACAAGACCCGCAACCAGGACGAGGGGATCCTGCCCTCGGGCAGACGGGG  
CACGGCGAGAGGTCTCTGCCAGA

&gt;&lt;stop&gt;

TAAGCTGTAGGGGCTCAGGCCACCCTCCCTGCCACGTGGAGACGCAGAGGCCGAACCCAA  
ACTGGGGCCACCTCTGTACCCTCACTTCAGGGCACCTGAGCCACCCTCAGCAGGAGCTGG  
GGTGGCCCCCTGAGCTCCAACGGCCATAACAGCTCTGACTCCACGTGAGGCCACCTTTGG  
GTGCACCCCACTGGGTGTGTGTGTGTGTGTGAGGGTTGGTTGAGTTGCCTAGAACCCCTG  
CCAGGGCTGGGGGTGAGAAGGGGAGTCATTACTCCCCATTACCTAGGGCCCCCTCCAAAAG  
AGTCCTTTTAAATAAATGAGCTATTTAGGTGCTGTGATTGTGAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIG. 5

SEQ ID NO:8

&lt;subunit 1 of 1, 422 aa, 1 stop

&lt;MW: 46302, pI: 9.42, NX(S/T): 6

< 1 10 20 30 40 50 60 70 80  
 < MPAGRRGPAAQSAARRPPPLPLLLLCVLCAPRAGSGAHTAVISPDPTLLIGSSLLATCSVHGDPGATAEGLYWTLNG  
 < 81 90 100 110 120 130 140 150 160  
 < RRLPPELSRVLNASTLALANLNGSRQSGDNLVCHARDGSILAGSCLYVGLPPEKPVNISCSKNMKDLTCRWTPGAH  
 < 161 170 180 190 200 210 220 230 240  
 < GETFLHTNYSLYKLRWYGQDNTCEEYHTVGPHSCHIPKDLALFTPYEIWVEATNRLGSARSDVLTLDILDVVTTDPPPD  
 < 241 250 260 270 280 290 300 310 320  
 < VHVS RVGGLEDQLSVRWVSPALKDFLFQAKYQIRYRVEDSDWKVVDVSNQTSCLAGLKPGETVYFVQVRCNPFGIYG  
 < 321 330 340 350 360 370 380 390 400  
 < SKKAGIWSEWSHPTAASTPRSERPPGPGGACEPRGGEPPSSGPPVRRRELKQFLGWLKKHAYCSNLSFRLYDQWRAMQKSHK  
 < 401 410 420  
 < TRNQDEGILPSGRRGTARGPAR

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FIG. 6



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SEQ ID NO:7 1 GACAGTGGAG GGCAGTGGAG AGGACCGCGC TGCTCTGCTG TCACCAAGAG CTGGAGAGAC CATCTCCAC CGAGATCAT GGCCTATTG GCCCTGCACC  
CTGTACCTC CCGTCACCTC TCCTGGCGCG ACAGGACGAC ACTGGTTCTC GACTCTCTG GACCTCTG M A P L A L H L  
1  
201 CTATGAGCAG CTGCTCAAGG TGGTGACCTG GGGCTCAAT CGACCTGTA AGCCCTGAG GGTGATTTG GTTGGCGCTG GTGTGGCGG GCTGTGGGCC  
GATACTGTC GACGAGTTCC ACCACTGGAC CCCGAGTTA GCTGGGACT TCGGGTCTC CCACTAACAC CAACCGGAC CACACCGGCC CGACCCACCG  
42 Y E Q L L K V V T W G L N R T L K P Q R V I V V G A G V A G L V A  
201 GCAAAGTGC TCAGCATGC TGACACAAG GTACCATCC TGAGGCGA TAACAGGATC GGGGCGGCA TCTTCACCTA CCGGACCAG AACACGGCT  
CGTTCCACG AGTCGCTACG ACCTGTGTC CAGTGGTAG ACCTCGTCT ATTGTCTTAG CCCCCTGCT AGAAGTGGAT GGCCTGGTC TTGTGCCCGA  
75 A K V L S D A G H K V T I L E A D N R I G G R I F T Y R D Q N T G W  
401 GGATTGGGA GCTGGAGCC ATGCGCATGC CCAGCTCTCA CAGATCTC CACAAGCTCT GCCAGGGCTT GGGGCTCAAC CTGACCAAGT TCACCCAGTA  
CCTAACCCCT CGACCTCGG TAGCGTAGG GTTCGAGAGT GTCTAGGAG GTGTTCGAGA CGGTCCCGA CCCCAGTTG GACTGTTCA AGTGGTCTAT  
109 I G E L G A M R M P S S H R I L H K L C Q G L G L N L T K F T Q Y  
501 CGACAAGAC ACGTGGACG AGGTGCACGA AGTGAAGTG CGCAACTATG TGTTGGAGAA GGTGCGCGAG AAGCTGGCT ACGCTTGGC TCCCGAGAA  
GCTGTTCTTG TGCACCTGCC TCACCTGCT TCACTTCGAC GCGTGTATAC ACCACTCTT CCACGGGCTC TTCGACCGA TCGGAGACG AGGGTCTCT  
142 D K N T W T E V H E V K L R N Y V V E K V P E K L G Y A L R P Q E  
601 AAGGGCACT GCGCGAAGA CATCTACCAG ATGCTCTCA ACCAGGCCCT CAAAGACCTC AAGCACTGG CCTGCAGAA GCGATGAAG AAGTTGAAA  
TTCCCGTGA GCGGGCTTCT GTAGATGGTC TACCGAGAGT TGGTCCGGA GTTCTCTGAG TTCCGTGACC CGACTCTTT CCGTACTTC TTCAACTTT  
175 K G H S P E D I Y Q M A L N Q A L K D L K A L G C R K A H K K F E R  
701 GGCACAGCT CTGGAATAT CTCTCGGG AGGGAACCT GAGCGGCGG GCGTGCAGC TTCTGGAGA CGTGTGTCC GAGGATGGCT TCTTCTATCT  
CCGTGTGGA GAACCTTATA GAAGAGCCCC TCCCTTGA CTCGCGCGC CGGACGTCG AAGACCTCT GCCTACAGG CTCCTACCGA AGAAGATAGA  
209 H T L L E Y L L G E G N L S R P A V Q L L G D V M S E D G F F Y L

SEQ ID NO:8<sup>met</sup>  
M A P L A L H L

FIG. 7A

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801 CAGTCCTGCC GAGGCCTCC GGGCCACAG CTGCTCAGC GACAGACTCC AGTACAGCCB CATCGTGGGT GGCTGGGACC TGCTGGCGCG CGCGCTGCTG  
 242 GTCGAAGCGG CTCCGGGAGG CCGGGGTGC GACGGAGTCG CTGTCTGAGG TCATGTGGGC GTAGCACCCA CCACCTCTGG ACAGCGCGC GCGGACGAC  
 242 S F A E A L R A H S C L S D R L Q Y S R I V G G W D L L P R A L L  
 901 AGTCGCTGT CCGGCTGTG CTGTGTAAC GCGCCCTGG TGCGATGAC CCAGGAGCCG CAGCATGTGC ACGTGCAGAT CGAGACTCT CCCCCGGCG  
 TCGAGGACA GGGCCGAAACA CGACAACCTG CCGGGGACC ACCGCTACTG GGTCCCTGGC GTCTACAGB TGCACGTCTA GCTCTGGAGA GGGGCGCGG  
 275 S S L S G L V L L N A P V V A M T Q G P H D V H V Q I E T S P P A R  
 1001 GGAATCTGAA GGTGCTGAAG GCCGACGTGG TGCTGTGAC GCGGAGCGGA CCGCGGTGA AGCGCATCAC CTCTCGCGG CCGTGCACC GCCACATGCA  
 CCTTAGACTT CCACGACTTC CGGTGCACC AGCAGACTG CCGCTCGCT GCGCGCCTT TCCGCTAGTG GAAGAGCGGC GGCACGCGG CGGTGTACGT  
 309 N L K V L K A D V V L L T A S G P A V K R I T F S P P L P R H M Q  
 1101 GGAGCGCTG CGAGGCTGC ACTACGTGC GCCACCAAG GTGTTCTTA GCTTCCGAG GCCTCTTGG CCGAGGAGC ACATTGAAG CGGCCACTCA  
 CCTCCGAC GCCTCCGACG TGATGACGG CCGGTGCTT CACAAGGATT CGAAGCGTC CGGAAGGACC GCGCTCTCTG TGTAACTTCC GCCGTGAGT  
 342 E A L R R L H Y V P A T K V F L S F R R P F W R E H I E G G H S  
 1201 AACACCGATC GCCGTGCGG CATGATTTT TACCCTCCG CCGCGGAGG CCGCTGCTG CTGGCTCTGT ACACGTGTC GGACGCGCG GCACGCTTCG  
 TTGTGGCTAG CCGGAGCGG GTACTAAAG ATGGCGCG ATGGCGCG GCGCGTCCC GCGGACGAC GACCGGAGA TGTGCACCAG CCTCGCGCG CGTCCGACG  
 375 N T D R P S R M I F Y P P P R E G A L L L A S Y T W S D A A A A F A  
 1301 CCGGCTTGG CCGGAAGAG CGTTGCGCT TGGCGTGA CGACGTGCG GCAATGACG GCGCTGCTGT GCGCCAGCTC TGGACGGCA CCGGCTCTG  
 GGCGAACTC GGCCCTCTC CGCAACGCA ACCGCGAGT GGTGACGCG CTAACGTC CCGACAGCA CCGGCTGAG ACCCTGCGT GCGCGCAGCA  
 409 G L S R E E A L R L A L D D V A A L H G P V V R Q L W D G T G V V  
 1401 CAAGCGTTG GCGGAGGACC AGCAGGCA GGTGCTTT GTGGTACAG CCGCGCGCT CTGGCAACC GAAAAGGATG ACTGACGCT CCCTTATGG  
 GTTCGCAACC CGCTCTCTG TCGTGTGGT CCCACGAAA CACCATGTC GCGCGCGGA GACGTTTGG CTCTTCTTAC TGACCTGCCA GGAATACCG  
 442 K R W A E D Q H S Q G G F V V Q P P A L W Q T E K D D W T V P Y G  
 1501 CGCATCTACT TTGCGGCGA GCACACGCC TACCAGCAG GCTGGTGA GACGGCGTC AAGTCGGCG TGCGCGCGC CATCAAGATC AACAGCCGA  
 GCGTAGATGA AACGGCGCT CGTGTGCGG ATGGCGTGC CGACCCACT CTGCGCGCAG TTGAGCCCG ACOCGCGCG GTAGTTCTAG TTGTGCGCT  
 475 R I Y F A G E H T A Y P H G W V E T A V K S A L R A A I K I N S R K  
 1601 AGGGGCTGC ATCGACACG GCCAGCCCC AGGGGACGC ATCTGACATG GAGGGGAGG GGCATGTGCA TGGGCTGGCC AGCAGCCCT CGCATGACCT  
 TCCCGGACG TAGCTGTGC CGGTGCGGC TCCCGTGC TAGACTGTAC CTCCCGTCC CGGTACAGT ACCCCACCG TCGTGGGGA GGTACTGGA  
 509 G P A S D T A S P E G H A S D M E G Q G H V H G V A S S P S H D L  
 1701 GGCAAGGAA GAAGCAGCC ACCCTCCAGT CCAAGCCAG TTATCTCTC AAACACGAC CCACACGAG ACCTCGCATT AAAGTATTT CGGAAAAA  
 CCGTTCTCTT CTTCGTCG TGGAGGTCA GGTTCGGTC AATAGAGG TTTGTGCTG GGTGTGCTC TGGAGCGTAA TTTCATAAAA GCCTTTTTT  
 542 A K E E G S H P P V Q G Q L S L Q N T T H T R T S H O  
 1801 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA AAAA  
 TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTT

FIG. 7B

**SEQ ID NO:8**

## FEATURES:

**pI: 8.97**

**Signal peptide: 1-21 (MAPLALHLLVLVPILLSLVAS)**

**Potential N-glycosylation sites: 54-57 (NRTL), 134-137 (NLTK), 220-223 (NLSR), and 559-562 (NTTH)**

**D-amino acid oxidases protein sequence identity: 61-80 (RVIVVGAGVAGLVAAKVLSD)**

FIG. 8

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## SEQ ID NO:9

GACTAGTTCTCTTGGAGTCTGGGAGGAGGAAAGCGGAGCCGGCAGGGAGCGAACCAGGAC  
TGGGGTGACGGCAGGGCAGGGGGCGCCTGGCCGGGGAGAAGCGCGGGGGCTGGAGACCA  
CCAAGTGGAGGGTCCGGAGTAGCGAGCGCCCCGAAGGAGGCCATCGGGGAGCCGGGAGGG  
GGGACTGCGAGAGGACCCCGGCGTCCGGGCTCCCGGTGCCAGCGCT  
><MET {trans=1-s, dir=f, res=1}  
ATGAGGCCACTCCTCGTCCTGCTGCTCCTGGGCCTGGCGGCCGGCTCGCCCCACTGGAC  
GACAACAAGATCCCCAGCCTCTGCCCCGGGGCACCCCGGCCTTCCAGGCACGCCGGGGCCAC  
CATGGCAGCCAGGGCTTGCCGGGCGCGATGGCCGCGACGGCCGCGACGGCGCGCCCCGGG  
GCTCCGGGAGAGAAAGGCGAGGGCGGGAGGCCGGGACTGCCGGGACCTCGAGGGGACCCC  
GGGCCGCGAGGAGAGGCGGGACCCGCGGGGCCACCGGGCCTGCCGGGGAGTGCTCGGTG  
CCTCCGCGATCCGCCTTCAGCGCCAAGCGCTCCGAGAGCCGGGTGCCTCCGCCGTCTGAC  
GCACCCTTGCCCTTCGACCGCGTGCTGGTGAACGAGCAGGGACATTACGACGCCGTCAAC  
GGCAAGTTCACCTGCCAGGTGCCTGGGGTCTACTACTTCGCCGTCCATGCCACCGTCTAC  
CGGGCCAGCCTGCAGTTTGATCTGGTGAAGAATGGCGAATCCATTGCCTCTTTCTTCCAG  
TTTTTCGGGGGGTGGCCCAAGCCAGCCTCGCTCTCGGGGGGGGCCATGGTGAGGCTGGAG  
CCTGAGGACCAAGTGTGGGTGCAGGTGGGTGTGGGTGACTACATTGGCATCTATGCCAGC  
ATCAAGACAGACAGCACCTTCTCCGGATTTCTGGTGTACTCCGACTGGCACAGCTCCCCA  
GTCTTTGCTTAGTGCCCACTGCAAAGTGAGCTCATGCTCTCACTCCTAGAAGGAGGGTGT  
GAGGCTGACAACCAGGTCATCCAGGAGGGCTGGCCCCCTGGAATATTGTGAATGACTAG  
GGAGGTGGGGTAGAGCACTCTCCGTCTGCTGCTGGCAAGGAATGGGAACAGTGGCTGTC  
TGCGATCAGGTCTGGCAGCATGGGGCAGTGGCTGGATTCTGCCCAAGACCAGAGGAGTG  
TGCTGTGCTGGCAAGTGTAAGTCCCCAGTTGCTCTGGTCCAGGAGCCCACGGTGGGGTG  
CTCTCTTCTGCTCCTCTGCTTCTCTGGATCCTCCCCACCCCTCCTGCTCCTGGGGCCG  
GCCCTTTTCTCAGAGATCACTCAATAAACCTAAGAACCCTCATAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAA

FIG. 9

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## SEQ ID NO:10

><subunit 1 of 1, 243 aa, 1 stop  
><MW: 25298, pI: 6.44,

<signal peptide>  
MRPLLVLALLGLAAG

<start of the mature protein>  
SPPLDDNKIPSLCPGHPGLPGTPGHHGSQGLPGRDGRDGRDGPAPGEKGE

<potential N-myristoylation site>  
GGRPGLPGPRGDPGPRGEAGPAGPTGPAGECSVPPRSAFSAKRSESRVPPPSDAPLPFDRVLVNEQGHYDAVTGKFTCQVPGVYYF  
AVHATVYRASLQFDLVKNGESIASFFQFFGGWPKPASLSGGAMVRLEPEDQVWVQVGVDYI

<potential N-myristoylation site>  
GIYASIKTDSTFSGFLVYSDWHSSPVFA

FIG. 10

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SEQ ID NO:11

GCATCGCCCTGGGTCTCTCGAGCCTGCTGCCTGCTCCCCGCCCCACCAGCC  
><MET {trans=1-s, dir=f, res=1}  
ATGGTGGTTTCTGGAGCGCCCCAGCCCTGGGTGGGGGCTGTCTCGGCACCTTCACCTCC  
CTGCTGCTGCTGGCGTCGACAGCCATCCTCAATGCGGCCAGGATACCTGTTCCCCCAGCC  
TGTGGGAAGCCCCAGCAGCTGAACCGGGTGTGGGCGGCAGGACAGCACTGACAGCGAG  
TGGCCCTGGATCGTGAGCATCCAGAAGAATGGGACCCACCACTGCGCAGGTTCTCTGCTC  
ACCAGCCGCTGGGTGATCACTGCTGCCCCTGTTTCAAGGACAACCTGAACAAACCATAC  
CTGTTCTCTGTGCTGCTGGGGGCTGGCAGCTGGGGAACCCTGGCTCTCGGTCCCAGAAG  
GTGGGTGTTGCCTGGGTGGAGCCCCACCCTGTGTATTCCTGGAAGGAAGGTGCCTGTGCA  
GACATTGCCCTGGTGCCTCTCGAGCGCTCCATACAGTTCTCAGAGCGGGTCTGCCCATC  
TGCCTACCTGATGCCTCTATCCACCTCCCTCCAAACACCCACTGCTGGATCTCAGGCTGG  
GGGAGCATCCAAGATGGAGTTCCTTGCCCCACCCTCAGACCCTGCAGAAGCTGAAGGTT  
CCTATCATCGACTCGGAAGTCTGCAGCCATCTGTACTGGCGGGGAGCAGGACAGGGACCC  
ATCACTGAGGACATGCTGTGTGCCGGCTACTTGGAGGGGGAGCGGGATGCTTGTCTGGGC  
GACTCCGGGGGGCCCCCTCATGTGCCAGGTGGACGGCGCCTGGCTGCTGGCCGGCATCATC  
AGCTGGGGCGAGGGCTGTGCCGAGCGCAACAGGCCCGGGGTCTACATCAGCCTCTCTGCG  
CACCGCTCCTGGGTGGAGAAGATCGTGCAAGGGGTGCAGCTCCGCGGGCGCGCTCAGGGG  
GGTGGGGCCCTCAGGGCACCGAGCCAGGGCTCTGGGGCCGCCGCGCTCCTAGGGCGCA  
GCGGGACGCGGGGCTCGGATCTGAAAGGCGGCCAGATCCACATCTGGATCTGGATCTGCG  
GCGGCCTCGGGCGGTTTCCCCCGCCGTAAATAGGCTCATCTACCTCTACCTCTGGGGGCC  
CGGACGGCTGCTGCGGAAAGGAAACCCCCCTCCCCGACCCGCCGACGGCCTCAGGCCCCC  
CTCCAAGGCATCAGGCCCCGCCCAACGGCCTCATGTCCCCGCCCCCAGACTTCCGGCCC  
CGCCCCCGGGCCCCAGCGCTTTTGTGTATATAAATGTTAATGATTTTTATAGGTATTTGT  
AACCCTGCCACATATCTTATTTATTCCTCCAATTTCAATAAATTATTTATCTCCAAAA  
AAAAAA

FIG. 11

SEQ ID NO:12

><signal peptide>  
MVVSGAPPALGGGCLGTFSTLLLLASTAILNA  
><start mature peptide>  
ARIPVPACGKPQQLNRVVGGEDSTDSEWPWIVSIQK  
><potential N-glycosylation site>  
NGTHHCAGSLLTSRWV  
><Serine proteases active site ITAAHC>  
ITAAHCFKDNLNKPYLFSVLLGAWQLGNPGSRSQKVGVAWVEPHPVYSWKEG  
ACADIALVRLERSIQFSERVLPICLPDASIHLPPNTHCWISGWGSIQDGVPLPHPQ  
TLQKLKVPIDSEVCSHLYWRGAGQGPITEDMLCAGYLEGERDACLGDSGGPL  
MCQVDGAWLLAGIISWGEGCAERNRPGVYISLSAHRSWVEKIVQGVQLRGRA  
QGGGALRAPSQGSGAAARS

FIG. 12

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SEQ ID NO:13

CTCTTTTGTCCACCAGCCCAGCCTGACTCCTGGAGATTGTGAATAGCTCCATCCAGCCTG  
AGAAACAAGCCGGGTGGCTGAGCCAGGCTGTGCACGGAGCACCTGACGGGCCCAACAGAC  
CC  
><MET {trans=1-s, dir=f, res=1}  
ATGCTGCATCCAGAGACCTCCCCTGGCCGGGGGCATCTCCTGGCTGTGCTCCTGGCCCTC  
CTTGGCACCACCTGGGCAGAGGTGTGGCCACCCAGCTGCAGGAGCAGGCTCCGATGGCC  
GGAGCCCTGAACAGGAAGGAGAGTTTCTTGCTCCTCTCCCTGCACAACCGCCTGCGCAGC  
TGGGTCCAGCCCCCTGCGGCTGACATGCGGAGGCTGGACTGGAGTGACAGCCTGGCCCAA  
CTGGCTCAAGCCAGGGCAGCCCTCTGTGGAATCCCAACCCCGAGCCTGGCATCCGGCCTG  
TGGCGCACCTTGCAAGTGGGCTGGAACATGCAGCTGCTGCCCGCGGGCTTGGCGTCCTTT  
GTTGAAGTGGTCAGCCTATGTTTTCAGAGGGGCAGCGGTACAGCCACGCGGCAGGAGAG  
TGTGCTCGCAACGCCACCTGCACCCACTACACGCAGCTCGTGTGGGCCACCTCAAGCCAG  
CTGGGCTGTGGGCGGCACCTGTGCTCTGCAGGCCAGACAGCGATAGAAGCCTTTGTCTGT  
GCCTACTCCCCCGGAGGCAACTGGGAGGTCAACGGGAAGACAATCATCCCCTATAAGAAG  
GGTGCCTGGTGTTCGCTCTGCACAGCCAGTGTCTCAGGCTGCTTCAAAGCCTGGGACCAT  
GCAGGGGGGCTCTGTGAGGTCCCCAGGAATCCTTGTGCGATGAGCTGCCAGAACCATGGA  
CGTCTCAACATCAGCACCTGCCACTGCCACTGTCCCCCTGGCTACACGGGCAGATACTGC  
CAAGTGAGGTGCAGCCTGCAGTGTGTGCACGGCCGGTTCCGGGAGGAGGAGTGCTCGTGC  
GTCTGTGACATCGGCTACGGGGGAGCCAGTGTGCCACCAAGGTGCATTTTCCCTTCCAC  
ACCTGTGACCTGAGGATCGACGGAGACTGCTTCATGGTGTCTTCAGAGGCAGACACCTAT  
TACAGAGCCAGGATGAAATGTCAGAGGAAAGGCGGGTGCTGGCCCAGATCAAGAGCCAG  
AAAGTGCAAGACATCCTCGCCTTCTATCTGGGCCGCTGGAGACCACCAACGAGGTGACT  
GACAGTGACTTCGAGACCAGGAACCTTCTGGATCGGGCTCACCTACAAGACCGCCAAGGAC  
TCCTTCCGCTGGGCCACAGGGGAGCACCAGGCCTTCACCAGTTTTGCCTTTGGGCAGCCT  
GACAACCACGGGCTGGTGTGGCTGAGTGCTGCCATGGGGTTTTGGCAACTGCGTGGAGCTG  
CAGGCTTCAGCTGCCTTCAACTGGAACGACCAGCGCTGCAAAACCCGAAACCGTTACATC  
TGCCAGTTTGCCCAGGAGCACATCTCCCGGTGGGGCCCAGGGTCCTGAGGCCTGACCACA  
TGGCTCCCTCGCCTGCCCTGGGAGCACCGGCTCTGCTTACCTGTCTGCCACCTGTCTGG  
AACAAGGGCCAGGTTAAGACCACATGCCTCATGTCCAAAGAGGTCTCAGACCTTGACAAA  
TGCCAGAAGTTGGGCAGAGAGAGGCGAGGAGGCGAGTGAGGGCCAGGGAGTGAGTGTTAG  
AAGAAGCTGGGGCCCTTCGCCTGCTTTTGATTGGGAAGATGGGCTTCAATTAGATGGCGA  
AGGAGAGGACACCGCCAGTGGTCCAAAAGGCTGCTCTCTTCCACCTGGCCCAGACCCTG  
TGGGGCAGCGGAGCTTCCCTGTGGCATGAACCCACGGGGTATTAAATTATGAATCAGCT  
GAAAAAAAAAAAAA

FIG. 13

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SEQ ID NO:14

&gt;&lt;homology to cysteine-rich secretory proteins&gt;

&gt;&lt;signal peptide&gt;

MLHPETSPGRGHLLAVLLALLGTTWA

&gt;&lt;start mature protein&gt;

EVWPPQLQEQAPMAGALNRKESFLLLSLHNRLRSWVQPPAADMRRLDWSDSLAQLA  
QARAALCGIPTPSLASGLWRTLQVGWNMQLLPAGLASFVEVVSLSLWFAEGQRYSHAA  
GECAR

&gt;&lt;potential N-glycosylation site&gt;

NATCTHYTQLVWATSSQLGCGRHLCSAGQTAIEAFVCAYSPGGNWEVNGKTIIPYKK  
GAWCSLCTASVSGCFKAWDHAGGLCEVPRNPCRMSCQNHGRL

&gt;&lt;potential N-glycosylation site&gt;

NISTCH

&gt;&lt;EGF-like domain cysteine pattern signature&gt;

CHCPPGYTGRYCQVRCSLQCVHGRFREEECS

&gt;&lt;EGF-like domain cysteine pattern signature&gt;

CVCDIGYGGAQCATKVHFPFHTCDLRIDGDCFMVSSEADTYRARMKCQRKGGVLA  
QIKSQKVQDILAFYLGRLETTNEVTDSDFETRNFWIGLTYKTAKDSFRWATGEHQAF  
TSFAFGQPDNHGLVWLSAAMGFGN

&gt;&lt;C-type lectin domain signature (CVELQASAAFNWNDQRCKTRNRYIC)&gt;

CVELQASAAFNWNDQRCKTRNRYICQFAQEHISRWGPGS

FIG. 14



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SEQ ID NO:15

GGGGCGGGTGGACGCGGACTCGAACGCAGTTGCTTCGGGACCCAGGACCCCCCTCGGGCCC  
GACCCGCCAGGAAAGACTGAGGCCGCGGCCCTGCCCCGCCCGGCTCCCTGCGCCGCCGCCG  
CCTCCCGGGACAGAAG

(ATG)TGCTCCAGGGTCCCTCTGCTGCTGCCGCTGCTCCTGCTACTGGCCCTGGGGCCTGGG  
GTGCAGGGCTGCCCATCCGGCTGCCAGTGCAGCCAGCCACAGACAGTCTTCTGCACTGCC  
CGCCAGGGGACCACGGTGCCCCGAGACGTGCCACCCGACACGGTGGGGCTGTACGTCTTT  
GAGAACGGCATCACCATGCTCGACGCAAGCAGCTTTGCCGGCCTGCCGGGCTGCAGCTC  
CTGGACCTGTACAGAACCAGATCGCCAGCCTGCGCCTGCCCCGCTGCTGCTGCTGGAC  
CTCAGCCACAACAGCCTCCTGGCCCTGGAGCCCGCATCCTGGACACTGCCAACGTGGAG  
GCGCTGCGGCTGGCTGGTCTGGGGCTGCAGCAGCTGGACGAGGGGCTCTTCAGCCGCTTG  
CGCAACCTCCACGACCTGGATGTGTCCGACAACCAGCTGGAGCGAGTGCCACCTGTGATC  
CGAGGCCTCCGGGGCTGACGCGCCTGCGGCTGGCCGGCAACACCCGCATTGCCCAGCTG  
CGCCCCGAGGACCTGGCCGGCCTGGCTGCCCTGCAGGAGCTGGATGTGAGCAACCTAAGC  
CTGCAAGGCCCTGCCTGGCGACCTCTCGGGCCTCTTCCCCCGCTGCGGCTGCTGGCAGCT  
GCCCCGAACCCCTTCAACTGCGTGTGCCCCCTGAGCTGGTTTGGCCCTGGGTGCGCGAG  
AGCCACGTACACTGGCCAGCCCTGAGGAGACGCGCTGCCACTTCCCGCCCAAGAACGCT  
GGCCGGCTGCTCCTGGAGCTTGACTACGCCGACTTTGGCTGCCAGCCACCACCACCA  
GCCACAGTGCCACCACGAGGCCCGTGGTGCGGGAGCCACAGCCTTGTCTTCTAGCTTG  
GCTCTACCTGGCTTAGCCCCACAGCGCCGGCCACTGAGGCCCCAGCCCGCCCTCCACT  
GCCCCACCGACTGTAGGGCCTGTCCCCCAGCCCCAGGACTGCCACCCTCCACTGCCTC  
AATGGGGGCACATGCCACCTGGGGACACGGCACCACCTGGCGTGCTTGTGCCCCGAAGGC  
TTCAGGGCCTGTACTGTGAGAGCCAGATGGGGCAGGGGACACGGCCAGCCCTACACCA  
GTCACGCCGAGGCCACCACGGTCCCTGACCCTGGGCATCGAGCCGGTGAGCCCCACCTCC  
CTGCGCGTGGGGCTGCAGCGCTACCTCCAGGGGAGCTCCGTGCAGCTCAGGAGCCTCCGT  
CTACCTATCGCAACCTATCGGGCCCTGATAAGCGGCTGGTGACGCTGCGACTGCCTGCC  
TCGCTCGCTGAGTACACGGTCACCCAGCTGCGGCCAACGCCACTTACTCCGTCTGTGTC  
ATGCCCTTTGGGGCCCGGGCGGGTGCCGAGGGGCGAGGAGCCTGCGGGGAGGCCCATACA  
CCCCAGCCGTCCACTCCAACACGCCCCAGTCACCCAGGCCCGGAGGGCAACCTGCCG  
CTCCTCATTTGCGCCCGCCCTGGCCGCGGTGCTCCTGGCCGCGCTGGCTGCGGTGGGGGCA  
GCCTACTGTGTGCGGCGGGGGCGGGCCATGGCAGCAGCGGCTCAGGACAAAGGGCAGGTG  
GGGCCAGGGGCTGGGCCCCTGGAACCTGGAGGGAGTGAAGGTCCCCCTGGAGCCAGGCCCG  
AAGGCAACAGAGGGCGGTGGAGAGGCCCTGCCCAGCGGGTCTGAGTGTGAGGTGCCACTC  
ATGGGCTTCCCAGGGCCTGGCCTCCAGTCACCCCTCCACGCAAAGCCCTACATCTAAGCC  
AGAGAGAGACAGGGCAGCTGGGGCCGGGCTCTCAGCCAGTGAGATGGCCAGCCCCCTCCT  
GCTGCCACACCACGTAAAGTTCTCAGTCCCAACCTCGGGGATGTGTGCAGACAGGGCTGTG  
TGACCACAGCTGGGGCCCTGTTCCCTCTGGACCTCGGTCTCCTCATCTGTGAGATGCTGTG  
GCCCAGCTGACGAGCCCTAACGTCCCCAGAACCAGTGCCATGAGGACAGTGTCCGCCC  
TGCCCTCCGCAACGTGCAGTCCCTGGGCACGGCGGGCCCTGCCATGTGCTGGTAACGCAT  
GCCTGGGGCCCTGCTGGGCTCTCCCACTCCAGGCGGACCCCTGGGGGCCAGTGAAGGAAGCT  
CCCGGAAAGAGCAGAGGGAGAGCGGGTAGGCGGCTGTGTGACTCTAGTCTTGGCCCCAGG  
AAGCGAAGGAACAAAGAACTGGAAAGGAAGATGCTTTAGGAACATGTTTTGCTTTTTT  
AAAATATATATATATTTATAAGAGATCCTTTCCCATTTATTTCTGGGAAGATGTTTTTCAA  
ACTCAGAGACAAGGACTTTGGTTTTTTGTAAGACAAACGATGATATGAAGGCCTTTTGTA  
GAAAAATAAAAAA

FIG. 15

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SEQ ID NO:16

MCSRVP L L L P L L L L L A L G P G V Q G C P S G C Q S Q P Q T V F C T A R Q G T T V P R D V P P D T V G L Y V  
F E N G I T M L D A S S F A G L P G L Q L L D L S Q N Q I A S L R L P R L L L L D L S H N S L L A L E P G I L D T A N V E  
A L R L A G L G L Q Q L D E G L F S R L R N L H D L D V S D N Q L E R V P P V I R G L R G L T R L R L A G N T R I A Q  
L R P E D L A G L A A L Q E L D V S N L S L Q A L P G D L S G L F P R L R L L A A A R N P F N C V C P L S W F G P W V  
R E S H V T L A S P E E T R C H F P P K N A G R L L L E L D Y A D F G C P A T T T T A T V P T T R P V V R E P T A L S S  
S L A P T W L S P T A P A T E A P S P P S T A P P T V G P V P Q P Q D C P P S T C L N G G T C H L G T R H H L A C L C P  
E G F T G L Y C E S Q M G Q G T R P S P T P V T P R P P R S L T L G I E P V S P T S L R V G L Q R Y L Q G S S V Q L R S L  
R L T Y R N L S G P D K R L V T L R L P A S L A E Y T V T Q L R P N A T Y S V C V M P L G P G R V P E G E E A C G E A  
H T P P A V H S N H A P V T Q A R E G N L P L L I A P A L A A V L L A A L A A V G A A Y C V R R G R A M A A A  
A Q D K G Q V G P G A G P L E L E G V K V P L E P G P K A T E G G G E A L P S G S E C E V P L M G F P G P G L Q S  
P L H A K P Y I

FIG. 16

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SEQ ID NO:17

GGCACTAGGACAACCTTCTTCCCTTCTGCACCACTGCCCCGTACCCTTACCCGCCCCGCCA  
CCTCCTTGCTACCCCACTCTTGAAACCACAGCTGTTGGCAGGGTCCCCAGCTC  
><MET {trans=1-s, dir=f, res=1}>  
ATGCCAGCCTCATCTCCTTTCTTGCTAGCCCCCAAAGGGCCTCCAGGCAACATGGGGGGC  
CCAGTCAGAGAGCCGGCACTCTCAGTTGCCCTCTGGTTGAGTTGGGGGGCAGCTCTGGGG  
GCCGTGGCTTGTGCCATGGCTCTGCTGACCCAACAAACAGAGCTGCAGAGCCTCAGGAGA  
GAGGTGAGCCGGCTGCAGGGGACAGGAGGCCCCCTCCAGAATGGGGAAGGGTATCCCTGG  
CAGAGTCTCCCGGAGCAGAGTTCCGATGCCCTGGAAGCCTGGGAGAATGGGGAGAGATCC  
CGGAAAAGGAGAGCAGTGCTCACCCAAAAACAGAAGAAGCAGCACTCTGTCCTGCACCTG  
GTTCCCATTAACGCCACCTCCAAGGATGACTCCGATGTGACAGAGGTGATGTGGCAACCA  
GCTCTTAGGCGTGGGAGAGGCCTACAGGCCCAAGGATATGGTGTCCGAATCCAGGATGCT  
GGAGTTTATCTGCTGTATAGCCAGGTCCTGTTTCAAGACGTGACTTTACCCATGGGTCAG  
GTGGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACTCTATTCCGATGTATAAGAAGTATG  
CCCTCCCACCCGGACCGGGCCTACAACAGCTGCTATAGCGCAGGTGTCTTCCATTTACAC  
CAAGGGGATATTCTGAGTGTCTAATTCCCCGGGCAAGGGCGAACTTAACCTCTCTCCA  
CATGGAACCTTCTGGGGTTTGTGAACTGTGATTGTGTTATAAAAAGTGGCTCCCAGCT  
TGGAAGACCAGGGTGGGTACATACTGGAGACAGCCAAGAGCTGAGTATATAAAGGAGAGG  
GAATGTGCAGGAACAGAGGCATCTTCTGGGTTTGGCTCCCCGTTCTCACTTTTCCCTT  
TTCATTCCCACCCCCTAGACTTTGATTTTACGGATATCTTGCTTCTGTTCCCCATGGAGC  
TCCG

FIG. 17

SEQ ID NO:18

<MW: 27433, pI: 9.85, NX(S/T): 2  
MPASSPFLAPKGPPEGVREPALSVLWLSWGAALGAVACAMALLTQQTELOSLRR  
EVSRLQGTGGPSQNGEGYPWQSLPEQSSDALEAWENGERSRKRRAVLTQKQKKQHSVLHL  
VPINATSKDDSDVTEVMWQPALRRGRGLQAQGYGVRIQDAGVYLLYSQVLFQDVTFTMGQ  
VVSREGQGRQETLFR CIRSMPSHPDRAYNSCYSAGVFHLHQGDILSVII PRARAKLNLSP  
HGTF LGFVKL

FIG. 18

SEQ ID NO:19 1 CGCGCGCGCG TCGCGCGCG AGGTGAGGG CGCGAGGTGA GGGCGCGAG GTTCCAGCA GGATGCCCGG GCTCTCCAGG AAGCTAAACT GAGAGGCCCG  
CGCGCGCGCG ACGCGCGCG TCCACTCCC CCGCTCCACT CCGCGGCTC CAAGGTCGT CCTACGGGCG CGAGACGTCC TTCGACTTCA CTCTCCGGCG

101 GAGAGGCCCG AGCCCGCCCG GGCAGGATG)ACCAAGGCC GGTGTTCGG CTTGTGGCTG GTGTGGGT CGGTGTCAT GATCTGCTG ATCATCTGT  
CTCTCCCGCG TCGCGCGCG CCGTCTAC TGGTTCGG CCGACAAGG CACACCGAC CACACCGCA GCCACAAGTA CTAGGACGAC TAGTAGCACA

1 SEQ ID NO:20 M T K A R L F R L W L V L G S V F M I L L I I V Y

201 ACTGGGACAG CGCAGCGCG GCGCACTTCT ACTGCACAC GTCTTCTCT AGCCCGACA CGGGCGCGG GCTGCCACG CCGGGCGCG ACAGGACAG  
TGACCTGTG CCGTCCCGG CCGGTGAAGA TGAACGTGT CAGGAAGAG TCGCGGTGT GCCCGGCGG CGACGGGTG GGGCCCGCG TGTCCCTGT

26 W D S A G A A H F Y L H T S F S R P H T G P P L P T P G P D R D R

301 GGAGCTCAG CCGACTCCG ATGTGACGA GTTCTGGAC AAGTTTCTCA GTCTGGCGT GAAGCAGAGC GACCTTCCCA GAAAGGAGC GGAGCAGCGC  
CCTCGAGTG CCGCTAGGC TACAGCTGCT CAAGACCTG TTCAAGAGT CACGACCGA CTTCGTCTCG CTGGAAGGT CTTCCTCTG CCTCGTCGGC

59 E L T A D S D V D E F L D K F L S A G V K Q S D L P R K E T E Q P

401 CCTCGCGCG GGAGCATGA GGAGGCTAG AGAGGCTAG ACTGTGCCC GCGCGACGC CAGACAGGC CCGCAGCAG CCGGACGCGA  
GGAGCGCGC CCTCGTACT CCTCTGCGC TCTCCGATG TGACGAGGG CCGCTGCGG CCGCGTCCC GTCTGGTCCC GCGCTGCTC CCGCTCGCCT

92 P A P G S M E E S V R G Y D W S P R D A R R S P D Q G R Q Q A E R R

501 GGAGCGTGT CCGGGCTTC TCGGCCAAT TCGCCTGCG CCGCTGCGC AAGAGCGCG CATTCGACGA CATCCCAAC TCGAGCTGA GCCACCTGAT  
CCTCGCACGA CGCCCGAAG ACGCGGTGA GGTGCGACG GAGGGGTG TTCTCGCGC GTAGCTGCT GTAGGGGTG AGCTCGACT CCGTGGACTA

126 S V L R G F C A N S S L A F P T K E R A F D D I P N S E L S H L I

601 CGTGGACGAC CGGCACGGG CCATCTACTG CTACGTGCC AAGTGGCT GCACCAACTG GAAGCGGTG ATGATCTGCG TGAGCGGAG CCTGTGAC  
GCACCTGTG CCGTCCCGG GOTAGATGAC GATGACGG TCCACCGA CGTGGTGC CTTCGCGCAC TACTAGCAG ACTGCCCTTC GAGCAGCGTG

159 V D D R H G A I Y C Y V P K V A C T N W K R V M I V L S G S L L H

701 CCGGTGCGC CCTACCGCA CCGCTGCGC ATCCCGCGC AGCAGTGA CAAGCCAGC CCGCACCTGA CCTTCAACA GTTCTGGCG CCGTACGGGA  
GCCCCACCG GATGGCGT GGGCGACCG TAGGCGCG TCGTGCAGT GTTGGGTG CCGTGGACT GGAAGTTGT CAAGACCGC CCGATGCCCT

192 R G A P Y R D P L R I P R E H V H N A S A H L T F N K F W R R Y G K

FIG. 19A

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801 AGCTCTCCCG CCACCTCATG AAGGTCAAGC TCAAGAAGTA CACCAAGTTC CTCTTCGTGC GCGACCCCTT ATCTCCGCTT TCOCGAGCAA  
 TCGAGAGGGC GGTGGAGTAC TTCCAGTTCC AGTCTTTCAT GTGGTTCAAG GAGAAACACG CGCTGGGGAA GCACGCGGAC TAGAGCGGA AGGCTCTGTT  
 226 L S R H L M K V K L K K Y T K F L F V R D P F V R L I S A P R S K  
 901 GTTCGAGCTG GAGAACGAGG AGTTCTACCG CAAGTTCCGC GTGCCATGC TCGGGCTGTA CGCAACACAC ACCAGCTGC CCGCTCCGGC GCGCAGGCGC  
 CAAGCTCGAC CTCTTGCTCC TCAAGATGCG GTTCAAGCGG CACGGGTACG ACGCCACAT CGGTTGGTG TGGTCGACG GCGGAGCCG CCGCTCCGGC  
 259 F E L E N E E F Y R K F A V P H L R L Y A N H T S L P A S A R E A  
 1001 TTCGGGCTG GCTCAAGGT GTCTTCGCC AACTTCATCC AGTACCTGCT GGACCCGAC ACGGAGAAGC TGGGCCCTT CAACGAGCAC TGGCGGACAG  
 AAGGCGGAC CGGAGTTCCA CAGGAAGCGG TTGAAGTAGG TCATGGACGA CCTGGGCTG TGCCTCTTCG ACCGCGGAA GTTGTCTGTG ACCGCGCTCC  
 292 F R A G L K V S F A N F I Q Y L L D P H T E K L A P F N E H W R Q V  
 1101 TGTACCGCT CTGCCACCG TGCAGATCG ACTACGACTT CGTGGGAAG CTGGAGACTC TGGACGAGGA CGCGCGGCG CTGCTGCAGC TACTCCAGGT  
 ACATGGCGGA GACGTTGGC ACGGTCTAGC TGATGCTGAA GCACCCCTTC GACCTCTGAG ACCTGCTCCT GCGGCGGCTC GACGACGTCG ATGAGGTCCA  
 326 Y R L C H P C Q I D Y D F V G K L E T L D E D A A Q L L Q L L Q V  
 1201 GGACCGGCG CTCCGCTTCC CCGGAGCTA CCGAACAGG ACCGCCAGCA GCTGGGAGGA GGACTGGTTC GCCAAGATCC CCCTGGCTG GAGGAGCAG  
 CCTGGCCGTC GAGGCGAAG GGGCTCGAT GGCCTTGCTC TGGCGTCTG CACCTCTCTT CCTGACCAAG CGGTTCTAGG GCGACCGGAC CTCCGTCGTG  
 359 D R Q L R F P P S Y R N R T A S S W E D W F A K I P L A W R Q Q  
 1301 CTGTATAAC TCTACGAGC CGACTTTGTT CTCTTCGGCT ACCCAAGCC CGAAACCTC CTCGAGACT GAAAGCTTTC GCGTTGCTTT TTCTCGGCTG  
 GACATATTG AGATGCTCC GCTGAAACAA GAGAAGCCGA TGGGGTTCCG GCTTTTGGAG GAGGCTCTGA CTTTCGAAAG CCGAACGAAA AAGAGCGCAC  
 392 L Y K L Y E A D F V L F G Y P K P E N L L R D O  
 1401 CCTGGAACT GACGACGCG CACTCCAGTT TTTTATGAC CTACGATTTT GCAATCTGG CTCTTGTTC ACTCCACTGC CTCTATCCAT TGAGTACTGT  
 GGAACCTTGA CTGGTGGC GTGAGGTCAA AAAAATCTG GATGCTAAA CATTAGACC GAGAACCAAG TGAGGTGACG GAGATAGGTA ACTCATGACA  
 1501 ATCGATATTG TTTTAAAGA TTAATATATT TCAGGTATTT AATACGA  
 TAGCTATAAC AAAAAATTCT AATTATATAA AGTCCATAAA TTATGCT

FIG. 19B

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SEQ ID NO:20  
<subunit 1 of 1, 414 aa, 1 stop  
<MW: 48414, pI: 9.54, NX(S/T): 4  
MTKARLFRLWLVLGVSVMILLIIVYWDSAGAAHFYLHTSFSRPHTGPPLPTPGPDRDREL  
TADSDVDEFLDKFLSAGVKQSDLPRKETEQQPPAPGSMEESVRGYDWSPRDARRSPDQGRQ  
QAERRSVLRGFCANSSLAFTPKEAFDDIPNSELHLIVDDRHGAIYCYVPKVACTNWKR  
VMIVLSGSLHLRGAPYRDPLRIPREHVHNASAHLTFNKFWRRYGKLSRHLMKVKLKKYTK  
FLFVRDPFVRLISAFRSKFELNEEFYRKFAVPMLRLYANHTSLPASAREAFRAGLKVSF  
ANFIQYLLDPHTEKLAPFNEHWRQVYRLCHPCQIDYDFVGKLETLDDEAAQLQLLQVDR  
QLRFPPSYRNRTASSWEEDWFAKIPLAWRQQLYKLYEADFVLFGYPKPENLLRD

**Signal peptide:**

1-31 MTKARLFRLWLVLGVSVMILLIIVYWDSAGA

**N-glycosylation site.**

134 NSSL  
209 NASA  
280 NHTS  
370 NRTA

TNFR/NGFR family cysteine-rich region protein  
329 CHPC

**FIG. 20**

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SEQ ID NO:21 1 CACCCCTCCA TTTCTGCGCA TGGACCCCTGC ACTGTCCTG ATCCCTGCTG CCTCGCCTC TTTCATCTG GCCTTGGCA CCGAGTGA GTTCGTGCG  
GTGGGAGGT AAGAGCGT ACGGGGACG TGACGAGGAC TAGGACGAC GGGAGCGAG AAGTAGGAC CGGAACCGT GGCCTCACCT CAAGCAGCG  
1 SEQ ID NO:22 M A P A L L L I P A A L A S F I L A P G T G V E P V R

101 TTACCTCC TTGGCCACT TCTTGGAGG ATCCCGAGT CTGCTGCTC GATGCCGC CAGGATGCG TGGCTGCCCT GCAGGACCG AGCATCTCTG  
AAATGGAGG AAGCCGTGA AGAACCTCC TAGGGCTCA GACCACGAG CCTACGGCG GTCCCTACCG ACCGACGGA CTTCTGCG TCGTAGGAAC  
28 F T S L R P L L G G I P E S G G P D A R Q G W L A A L Q D R S I L A

201 CCCCCCTGC ATGGATCTG GGGCTCTGC TTCTATTGT TGGGACGAC AGCTCATG CAGCTGAAAG AGTGAAAGCA TGGACATCC GGTACTTTGG  
GGGGGACCG TACCTTAGC CCGGAGGAC AGATAAACA ACCGCTGCTG TCGAGTACC GTGACTTTC TCACCTTCCGT ACCTGTAGG CCATGAAACC  
62 P L A W D L G L L L L F V G Q H S L M A A E R V K A W T S R Y P G

301 GGTCTTCTAG AGTCACTGT ATGTGGCTG CACTGCTCTG GCCTGCGC TGGTGATCG GTACTGGGAG CCCATACCA AAGGCCCTGT GTTGTGGAG  
CCAGGAAGTC TCCAGTGACA TACACCGGAC GTGACGGGAC CGGAACGTCG ACCACTAGC CATGACCTC GGTATGCGT TTCCGGGACA CAACACCTC  
95 V L Q R S L Y V A C T A L A L Q L V M R Y W E P I P K G P V L W E

401 GCTGGGCTG AGCATGGGC CACTGGGTG CCGCTCTCT GCTTGTGCT CCATGTCATC TCTGGCTCC TCATCTTAG CATCTTCTC GTCTTTGACT  
CGAGCCCGAC TCGGTACCG GTGACCCAC GCGAGGAGA CGAACACGA GTACACGAG AGGACCGAG AGTAGAAATC GTAGGAAGAG CAGAAACTGA  
128 A R A E P W A T W V P L L C F V L H V I S W L L I F S I L L V F D Y

501 ATGCTGAGT CATGGGCTC AAGCAGGTAT ACTACATGT GCTGGGCTG GCGAGCTC TGGCCCTGAA GTCTCCCGG GCTCTCAGAC TCTTCTCCA  
TACGACTCGA GTACCCGAG TTTGTCCATA TGATGGTACA CGACCCGAC CCGCTCGAG ACCGGACTT CAGAGGGCC CGAGATCTG AGAAGAGGT  
162 A E L M G L K Q V Y Y H V L G L G E P L A L K S P R A L R L F S H

601 CTTGCGCCAC CCAGTGTGT TGGAGCTGT GACAGTGTG TGGTGTGCT CTACCTGG CACGACCGT CTCTCTCTG CTTTCTCTT TACCTCTAC  
GGACCGGTG GTTCACACAC ACCTGACGA CTGTACGAC ACCCACGAC GATGGACCG GTGCTGGCA GAGGAGAAC GAAAGGAGA ATGGAGATG  
195 L R H P V C V E L L T V L W V V P T L G T D R L L L A F L L T L Y

701 CTGGGCTTG CTCACGGGT TGATCAGCA GACCTCCGT ACCTCGGGC CCAGCTACA AGAAACTCC ACTGCTCTC TCGGCCCG GATGGGAGG  
GACCGGACC GATGCCCGA ACTAGTGTG TGGAGGCGA TGGAGGCG GTGATGTT TCTTTGAGG TGACGAGAG AGCCGGGTG CTACCTCTC  
228 L G L A H G L D Q Q D L R Y L R A Q L Q R K L H L L S R P Q D G E A

FIG. 21A

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801 CAGAGTGG AGCTCACTCT GGTACAAAG CCTGTTCTTC CTCTCCCACT GAATTTAA TCTTAAACAT CCAGGCCCTG GCTGCTTCAT GCCAGAGGCC
GTCTCACTCC TCGAGTGAGA CCAATGTTCC GACAAAGAAC GAGAGGTGA CTTAAGATTT AGGAATTOTA GGTCCGGGAC CGACGAAGTA CGGTCTCCGG
262 E O

901 CAAATCCATG GACTGAAGGA GATGCCCTTT CTACTACTTG AGACTTTATT CTCTGGTCC AGCTCCATAC CCTAAATTTCT GAGTTTCAGC CACTGAACTC
GTTTAGGTAC CTGACTTCTT CTACGGGA GATGATGAAC TCTGAATAA GAGACCCAGG TCGAGGTATG GGAATTTAAGA CTCAAAGTGG GTGACTTGAG

1001 CAAAGTCCAC TTCTCACCAG CAAGGAAGAG TGGGGTATGG AAGTCATCTG TCCCTTCACT GTTTAGAGCA TGACACTCTC CCCCTCAACA GCCTCCTGAG
GTTCCAGGTG AAGAGTGGTC GTTCTCTCTC ACCCCATACC TTCAGTAGAC AGGAAGTGA CAAATCTCOT ACTGTGAGAG GGGGAGTTGT CGGAGGACTC

1101 AAGGAAGGA TCTGCCCTGA CCACTCCCTT GGCATGTTA CTTGCTCTTG CGCTCAGGG GTCCCTTCTT GCACCGCTGG CTTCGACTCC AAGAAGGTGG
TTCTTTTCTT AGACGGGACT GGTGAGGGGA CCGTGACAAAT GAACGGAGAC GCGGAGTCCC CAGGGGAAGA CGTGCGGACC GAAGGTGAGG TTCTTCCACC

1201 ACCAGGTCT GCAAGTTCAA CGGTCAATGC TGTCCCTCCA GGCCCAACC TTGCTCACC ACTCCGGCC CTAGTCTCTG CACCTCCTTA GGCCCTGCCT
TGGTCCCAAG CGTTCAAAGTT GCCAGTATCG ACAGGGAGGT CCGGGGTGG ACGGAGTGG TGAGGGCCGG GATCAGAGAC GTGAGGAAT CGGAGACGGA

1301 CTGGGCTCAG ACCCCAACCT AGTCAGGGG ATTCTCCTGC TCTTAACTCG ATGACTTGG GCTCCCTGCT CTCCCGAGGA AGATGCTCTG CAGGAAATA
GACCCGAGTC TGGGTTGA TCAGTTCCC TAAGAGGACG AGAATTGAGC TACTGAACCC CGAGGACGA GAGGGCTCCT TCTACGAGAC GTCTTTTAT

1401 AAAGTCAGCC TTTTCTAAA AAAA
TTTCAGTCGG AAAAAGATTT TTTT

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FIG. 21B



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## SEQ ID NO:22

&lt;MW: 29379, pI: 8.93, NX(S/T): 0

MAPALLLIPAALASFILAFGTGVEFVRFTSLRPLLGGIPESGGPDARQGWLAAALQDRSIL  
APLAWDLGLLLLFVGQHSLSMAAERVKAWTSRYFGVLQRSLYVACTALALQLVMRYWEPIF  
KGPVLWEARAEPWATWVPLLCFVLHVISWLLIFSILLVFDYAELMGLKQVYYHVLGLGEP  
LALKSPRALRLFSLRHPVCVELLTVLWVPTLGTDRLLLAFLTLYLGLAHGLDQQDLR  
YLRAQLQRKLHLLSRPDGEAE

## Signal peptide:

1-13 MAPALLLIPAALA

## Transmembrane domain:

58-76 SILAPLAWDLGLLLLFVGQ  
99-113 SLYVACTALALQLVM  
141-159 CFVLHVISWLLIFSILLVF  
203-222 LLTVLWVPTLGTDRLLLAFL

FIG. 22

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SEQ ID NO:23

GCAATGGCGCTGAGTTCCTCTGCTGGAGTTCATCCTGCTAGCTGGGTTCCCGAGCTGCCG  
GTCTGAGCCTGAGGCATGGAGCCTCCTGGAGACTGGGGGCCTCCTCCCTGGAGATCCACC  
CCCAGAACCGACGTCTTGAGGCTGGTGCTGTATCTCACCTTCCTGGGAGCCCCCTGCTAC  
GCCCCAGCTCTGCCGTCTGCAAGGAGGACGAGTACCCAGTGGGCTCCGAGTGCTGCCCC  
AAGTGCAGTCCAGGTTATCGTGTGAAGGAGGCCTGCGGGGAGCTGACGGGCACAGTGTGT  
GAACCCTGCCCTCCAGGCACCTACATTGCCACCTCAATGGCCTAAGCAAGTGTCTGCAG  
TGCCAAATGTGTGACCCAGCCATGGGCCTGCGCGGAGCCGGAAGTGTCTCCAGGACAGAG  
AACGCCGTGTGTGGCTGCAGCCCAGGCCACTTCTGCATCGTCCAGGACGGGGACCACTGC  
GCCGCGTGCCGCGCTTACGCCACCTCCAGCCCCGGGCCAGAGGGTGCAGAAGGGAGGCACC  
GAGAGTCAGGACACCCTGTGTCAGAACTGCCCCCGGGGACCTTCTCTCCCAATGGGACC  
CTGGAGGAATGTCAGCACCCAGACCAAGTGCAGCTGGCTGGTGACGAAGGCCGGAGCTGGG  
ACCAGCAGCTCCCACTGGGTATGGTGGTTTCTCTCAGGGAGCCTCGTCATCGTCATTGTT  
TGCTCCACAGTTGGCCTAATCATATGTGTGAAAAGAAGAAAGCCAAGGGGTGATGTAGTC  
AAGGTGATCGTCTCCGTCCAGCGGAAAAGACAGGAGGCAGAAGGTGAGGCCACAGTCATT  
GAGGCCCTGCAGGCCCCCTCCGGACGTCACCACGGTGGCCGTGGAGGAGACAATACCCTCA  
TTCACGGGGAGGAGCCCAAACCACTGA

FIG. 23

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SEQ ID NO:24

met glu pro pro gly asp trp gly pro pro pro trp arg ser thr  
pro arg thr asp val leu arg leu val leu tyr leu thr phe leu  
gly ala pro cys tyr ala pro ala leu pro ser cys lys glu asp  
glu tyr pro val gly ser glu cys cys pro lys cys ser pro gly  
tyr arg val lys glu ala cys gly glu leu thr gly thr val cys  
glu pro cys pro pro gly thr tyr ile ala his leu asn gly leu  
ser lys cys leu gln cys gln met cys asp pro ala met gly leu  
arg ala ser arg asn cys ser arg thr glu asn ala val cys gly  
cys ser pro gly his phe cys ile val gln asp gly asp his cys  
ala ala cys arg ala tyr ala thr ser ser pro gly gln arg val  
gln lys gly gly thr glu ser gln asp thr leu cys gln asn cys  
pro pro gly thr phe ser pro asn gly thr leu glu glu cys gln  
his gln thr lys cys ser trp leu val thr lys ala gly ala gly  
thr ser ser ser his trp val trp trp phe leu ser gly ser leu  
val ile val ile val cys ser thr val gly leu ile ile cys val  
lys arg arg lys pro arg gly asp val val lys val ile val ser  
val gln arg lys arg gln glu ala glu gly glu ala thr val ile  
glu ala leu gln ala pro pro asp val thr thr val ala val glu  
glu thr ile pro ser phe thr gly arg ser pro asn his

FIG. 24

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SEQ ID NO:26 1 CGCCTCCGCC TTCGAGGCT GACCGCCCG GCGCCGTTT CAGGCTCTG CAGGCGGAT CGCAGCCGC CTGGCGGCG TCCAGGGCGG TCGGGGCGCT  
CGGAGGCGG AAGCTCCGA CTGGCGGGC CCGGGCAAG GTCCGGACAC GTCCGCGCTA GCGTCGGCG GACCGCGCT AGTCCCGCC ACGCCCGGA

101 GGGCGGGAGC CGGAGGCGC GGCAGGCATG GAGGCGCTG TGCTGGGCG GGGTGGCTG CTGGCGCTT ACGTCTTGT CTACTACAAC CTGGTGAAGG  
CCCCCGCTG GCGCTCGCG CCGCGCTAC CTCCGCGAG ACGACCGCG CCGGACGAA GACCGCGAA TGCACGAACA GATGATGTTG GACCACCTCC

1 SEQ ID NO:26 M E A L L L G A G L L L G A Y V L V Y Y N L V K A  
^MET

201 CCGCGCCGTG CCGGCGCATG GGCACCTGC GGGCGCGAC GCGCGTGTG CCGCGCGCA ACAGCGGCT CGGAAAGATG ACGGCGCTG AGTGGCGCG  
GGGCGGAC CCGCGCTAC CCGTGGACG CCGCGCGTG CCGCGCGTG CCGGACCGA TGCCCGGCTA GCTTCTTAC TGCCGCGAC TCGACCGCG

26 P P C G G M G N L R G R T A V V T G A N S G I G K M T A L E L A R  
^start 119 bp not in From DNA 43050

301 CCGGGAGCG CCGTGTGTC TGCCCTGCG CAGCAGGAG CCGGGGAGG CCGTGCCTT CGACCTCCG CAGGAGAGT GGAACAATGA GGTATCTTC  
GGCCCTCG CCGACCGAC ACCGACCGC GTCCGTCTC GCGCGCTCC CCGCGCGAA GCTGGAGCG GTCTCTCTAC CTTGTACT CCAGTAGAAG

59 R G A R V V L A C R S Q E R G E A A F D L R Q E S G N N E V I F  
^end 119 bp not in From DNA43050

401 ATGGCTTGG ACTTGGCCAG TCTGGCTCG GTGGGCGCT TGCCACTGC CTTCTGAGC TCTGAGCCAC GGTGGACAT CCTATCCAC AATGCCGTA  
TACCGGAACC TGAACGCTC AGACCGGAC CAGCGCGGA AACGCTGAC GAAAGACTCG AGACTCGTG CCAACTGTA GGAGTAGTG TTACGGCGCAT

92 M A L D L A S L A S V R A F A T A F L S S E P R L D I L I H N A G I

501 TCAGTTCCTG TGGCCGACC CGTGGGCGT TTAACCTGCT GCTTCGGTG AACCATATG GTCCCTTCT GCTGACACAT CTGCTGCTG CTTGCCGTA  
AGTCAAGGAC ACCGGCTCG GCATCCGCA AATTGGACGA CGAAGCCAC TTGGTATAGC CAGGAAAGA CGACTGTGTA GACGACGACG GAACGGACTT

126 S S C G R T R E A F N L L L R V N H I G P F L L T H L L L P C L K

601 GGCATGTGC CTAAGCGCG TGGTGTGTT AGCTCAGCT GCCACTGTC GGGAGCTCT TGACTTCAA CGCTGGACC GCCAGTGGT GGGCTGGCG  
CGTACACCG GATCGGCGC ACCACACCA TCGAGTCTGA TCGGAGTCTG CCGGTGACG CCGCTGACG ACTGAAGTTT GCGACCTG CCGGTACCA CCGACCGC

159 A C A P S R V V V A S A A H C R G R L D F K R L D R P V V G W R

701 CAGGAGTGC GGCATATGC TGACACTAAG CTGGCTAATG TACTGTTTG CCGGAGCTC GCCAACGAC TTGAGGCCAC TGGGTCACC TGCTATGAC  
GTCTCGAC CCGTATACG ACTGTGATC GACCGATTAC ATGACAAAC GCGCTCGAG CCGTGTGTCG AACTCCGCTG ACCGAGTGG ACGATACGTC

192 Q E L R A Y A D T K L A N V L F A R E L A N Q L E A T G V T C Y A A

801 CCCACCCAGG CCTGTGAAC TCGAGCTGT TCCTCGGCA TGTCTCTGA TGGTGGCC CACTTTTGG CCCATTGGCT TGGCTGTGC TCCGGGAC  
GGTGGTCC CCGACACTG AGCTCGACA AGGAGCGGT ACAAGGACCT ACCGACCGG GTGAAACGC GGTAAACCG ACCGACCG AGGCCGTGG

226 H P G P V N S E L F L R H V P G W L R P L L R P L A W L V L R A P

FIG. 25A

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901 AAGAGGGGT GCCAGACAC CCTGTATTG TGCTTACAA GAGGCATCG AGCCCTCAG TGGAGATAT TTGCCAACT GCCATGTGA AGAGTGCCT  
 TTCTCCCCA CGGTCTGTG GGGACATAAC ACGAGATGTT CTCCCGTAGC TCGGGAGTC ACCCTCTATA AACCGTTGA CGGTACACCT TCTCCACGGA  
 259 R G G A Q T P L Y C A L Q E G I E P L S G R Y F A N C H V E E V P  
 1001 CCAGCTGCC GAGACGACG GGCAGCCAT CGGCTATGG AGCCAGCAA GAGGCTGGC CTGGGAGGA TGGTGAACCC GATGAAGACC  
 GGTGACGGG CTCTGCTGC CCGTCGGTA GCCGATACCC TCCGTCGTT CTCGACCGT CCGGAACCG GACCCCTCT ACGACTTGG CTACTTCTGG  
 292 P A A R D D R A A H R L W E A S K R L A G L G P G E D A E P D E D P  
 1101 CCCAGTCTGA GGACTCAGAG GCCCATCTT CTCTAAGCAC CCCCACCCT GAGGAGCCCA CAGTTTCTCA ACCTTACCCC AGCCCTCAGA GCTCACCAGA  
 GGTTCAGACT CCGAGTCTC CGGGGTAGAA GAGATTCGT GGGGTGGGA CTCCTCGGT GTCAAGAGT TGGAAATGGG TCGGAGTCT CAGTGTCTT  
 326 Q S E D S E A P S S L S T P H P E E P T V S Q P Y P S P Q S S P D  
 1201 TTTGTCTAAG ATGACGCACC GAATTCAGG TAAAGTTGAG CCTGAGATCC AGCTCTCTA ACCCTCAGGC CAGATGCTT GCCATGGCAC TTCTATGTCTC  
 AAACAGATTC TACTGCGTGG CTTAAGTCCG ATTTCAACTC GGACTCTAGG TCGAGAGGAT TGGGAGTCCG GTCTACGAA CCGTACCCTG AAGTACCAGG  
 359 L S K M T H R I Q A K V E P E I Q L S O  
 1301 TTGAAGACCT CGGATGTGTG TGAGGCCATG CCCTGGACAC TGACGGGTTT GTGATCTGA CCTCCGTGTT TACTTCTTG GGCCCCAAGC TGTGCCCTGG  
 AACTTTTGA GCCTACACAC ACTCGGTAC GGGACCTGTG ACTGCCCAA CACTAGAAT GGAGGCACCA ATCAAGAGACC CCGGGTTTCG ACACGGGACC  
 1401 ACATCTCTTT TCCTGGTTGA AGGAATAATG GGTATTAAT TCTTCCTGAG AGTGACAGTA ACCCAGATG GAGAGATAGG GGTATGCTAG ACATGTGCT  
 TGTAGAGAAA AGGACCAACT TCCTTATTAC CCACTAATAA AGAAGACTC TCACTGTCTAT TGGGTCTTAC CTCTCTATCC CCATACGATC TGTGACACGA  
 1501 TCTCGGAAAT TTGGATGTAG TATTTTCAGG CCCCACCCTT ATTGATTCTG ATCAGCTCTG GAGCAGAGGC AGGGAGTTTG CAATGTGATG CACTGCCAAC  
 AGAGCCTTTA AACCTACATC ATAAAAGTCC GGGGTGGAA TAACTAAGAC TAGTCGAGAC CTCGTCTCCG TCCCTCAAAC GTTACACTAC GTGACGGTTG  
 1601 ATTGAGAATT AGTGAACCTGA TCCCTTTGCA ACCGTCTAGC TAGGTAGTTA AATTACCCC ATGTTAATGA AGCGGAATTA GGTCCCGAG CTAAGGGACT  
 TAACTCTTAA TCACTTGACT AGGAAACGT TGGCAGATCG ATCCATCAAT TTAATGGGG TACAATTACT TCGCCTTAAT CCGAGGGCTC GATTCCCTGA  
 1701 CGCCTAGGGT CTCACAGTGA GTAGGAGGAG GGCCTGGAT CTGAACCCAA GGTCTGAGG CCAGGCCGA CTGCCCTAAG ATGGTGTCTG AGAAGTGAGT  
 GCGGATCCCA GAGTGTCACT CATCTCTCTC CCGACCCCTA GACTTGGGT CCCAGACTCC GGTCCCGGT GACGCCATTC TACCCACGAC TCTTCACTCA  
 1801 CAGGGCAGGG CAGCTGGTAT CGAGGTGCCC CATGGGACTA AGGGGACGCC TTCCGGCGG ATGCAGGGCT GGGGTCTATCT GTATCTGAAG CCCCTCGGAA  
 GTCCCGTCCC GTCGACCATA GCTCCACGGG GTACCCCTCAT TCCCTCTCGG AAGGCCCGCC TAGCTCCGA CCCCACTAGA CATAGACTTC GGGAGGCCTT  
 1901 TAAAGGCGGT TGACCGCCAA AAAAAAAA AAAAAAAA  
 ATTTCCGCA ACTGCGGT TTTTTTTTT TTTTTTTTT

FIG. 25B

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## SEQ ID NO:26

MEALLLGALLGAYVLVYNNL VKAPCGMGNLRGRTAVVTGANSIGKMTALELARRG  
 ARVVLACRSQERGEAAAFDLRQESGNNEVIFMALD LASLASVRAFATAFLSSEPRLDILI  
 HNAGISSCGRTREAFNLLRVNHIGPFLLTHLLPCLKACAPSRVVVVASAAHCRGLDF  
 KRLDRPVVGWRQELRAYADTKLANVLFARELANQLEATGVTCTYAAHPGPVNSELFLRHVP  
 GWLRLRLRPLAWLVLAPRGGAQTPLYCALQEGIEPLSGRYFANCHVEEVPPAARDRAA  
 HRLWEASKRLAGLPGEDAEPDEDPQSEDSEAPSSLSTPHPEPTVSQPYSPQSSPDLS  
 KMTTHRIQAKVEPEIQLS

## Signal peptide:

amino acids 1-16

MEALLLGALLGAYV

## Glycosaminoglycan attachment site:

amino acids 46-49

SGIG

## Sequences typical of the short-chain alcohol dehydrogenase family:

starting at amino acid 37 RTAVVTGANSIG

starting at amino acid 114 PRLDILHNAG

FIG. 26

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SEQ ID NO:27 &lt; DNA58125 &gt;

GTGAAGGGAGCCGGGATCAGCCAGGGGCCAGC

&lt;met {trans=1-s, dir=f, res=1}&gt;

ATGAGCCGGAGGGAGGGAAGTCTGGAAGACCCCCAGACTGATTCCTCAGTCTCACTTCTT  
CCCCACTTGGAGGCCAAGATCCGTCAGACACACAGCCTTGCGCACCTCCTCACCAAATAC  
GCTGAGCAGCTGCTCCAGGAATATGTGCAGCTCCAGGGAGACCCCTTCGGGCTGCCCAGC  
TTCTCGCCGCCGCGGCTGCCGGTGGCCGGCCTGAGCGCCCCGGCTCCGAGCCACGCGGGG  
CTGCCAGTGACAGAGCGGCTGCGGCTGGACGCGGCGGCGCTGGCCGCGCTGCCCCGCTG  
CTGGACGCAGTGTGTGCGCCGCCAGGCCGAGCTGAACCCGCGCGCGCCGCGCCTGCTGCGC  
CGCCTGGAGGACGCGGCGCGCCAGGCCCGGGCCCTGGGCGCCGCGTGGAGGCCTTGCTG  
GCCGCGCTGGGCGCCGCCAACCGCGGGCCCCGGGCCGAGCCCCCGCCGCCACCGCCTCA  
GCCGCCTCCGCCACCGGGGTCTTCCCCGCCAAGGTGCTGGGGCTCCGCGTTTGCGGCCTC  
TACCGCGAGTGGCTGAGCCGCACCGAGGGCGACCTGGGCCAGCTGCTGCCCCGGGGCTCG  
GCCTGAGCGCCGCGGGGCAGCTCGCCCCGCCTCCTCCCGCTGGGTTCGCTCTCTCCTTCC  
GCTTCTTTGTCTTTCTCTGCCGCTGTGCGTGTCTGTCTGTCTGCTCTTAGCTGTCTCCAT  
TGCCCTCGGCCTTCTTTGCTTTTGTGGGGGAGAGGGGAGGGGACGGGCAGGGTCTCTGTC  
GCCCAGGCTGGGGTGCAGTGGCGCGATCCCAGCACTGCAGCCTCAACCTCCTGGGCTCAA  
GCCATCCTTCCGCCTCAGCTTCCCCAGCAGCTGGGACTACAGGCACGCGCCACCACAGCC  
GGCTAATTTTTTATTTAATTTTTTGTAGAGACGAGGTTTCGCCATGTTGCCAGGCTGGT  
CTTGAACCTCCGGGGCTCAAGCGATCCTCCCGCTTCAGCCTCCCTAAGTGCTGGGATTGCA  
GGCGTGAGCCACT

TTCCCAGCCTCTCTTTGCTTTGCC

TGCCCCGTTCTCTTAACTCTTGGACCCTCCTCGTCTGCATGGTAACTCCGTCTG

AGTCTACCATTTTTCTTGCTCTCCCTCCTTCCCTGGGGCTGCCTCAGTTCCCTTTGGCCTC  
CCCCTTTACCCAGCTCTTGGGGTGTCTCTGTTTTTTCCATCCCCACTTCCTGCCTTCTCG  
TGGCCCTGTGTGAGCACATGTGTACATCTCAGCCTTATCTCAAGGAGGTGACACCTTCTC  
TCCTTGTCCCCATCTGGCCGTCTCTGTGTCTTCCCTGGCCAGGGGCGTGCCTGCTGGTC  
CTATGGGGGGAAGGCTACTCCGCATCTCAGCCACCTTCCTCAGGCTCACTCCACCTACAT  
CCCCAGTCTGCCACACCCCATCCCTTTGGGCCTCAGCCCTGTCCCTTTGATGTCCTCCTT  
TCCTTCAGCCCCCTCTGCCCTGTCCCTGCACACCTCC

FIG. 27

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SEQ ID NO:28 &lt;p1.DNA58125&gt;

MSRREGSLEDPQTDSSVSLLPHLEAKIRQTHSLAHLTKYAEQLLQEYVQLQGDPFGLP  
SFSPRLPVAGLSAPAPSHAGLPVHERLRLDAAALAALPPLLDVCCRQAELNPAPRL  
LRRLEDAARQARALGAAVEALLAALGAANRGPRAEPPAATASAASATGVFPAKVLGLRV  
CGLYREWLSRTEGDLGQLLPGGSA

**FIG. 28**

SEQ ID NO:29 &lt;DNA28710&gt;

GTGGAGGGCGGGGGTGACAGCAGCCCGGAGCCGCGGAGCCTCAGCTTCCGCCTGGACCCA  
GCCTCGTGGGAGCCCCGCGGGTCCTGCCCAGATGTGGAAGACTGAGGCCTGTTGAAAGTG  
CAGAGCTCAGCCCTGGCACCCCTCTGTTCCCAAGAGCTCCATGCAGGTGCCACAGGATGGA  
GAAGACCTTGCTGGCCAACCTTGGTACCACGGCCTCCTGTCCCGCCAGAAGGCTGAAGCT  
CTTCTTCAGCAAAA

**FIG. 29**

SEQ ID NO:30 &lt;2452972H1&gt;

GGCTTANCCCCACAGAGCCGGCCACTGANGCCCCCAGCCCGCCCTCCACTGCCCCACCGA  
CTGTAGGGCCTGTCCCCCAGCCCCAGGACTGCCACCGTCCACCTGCCTCAATGGGGGCA  
CATGCCACCTGGGGACACGGCACCACTGGCGTGCTTGTGCCCCGAAGGCTTCACGGGCC  
TGTA CTGTGAGAGCCAGATGGGGCAGGGACACGGCCAGCCCTACACCAGTCA

**FIG. 30**



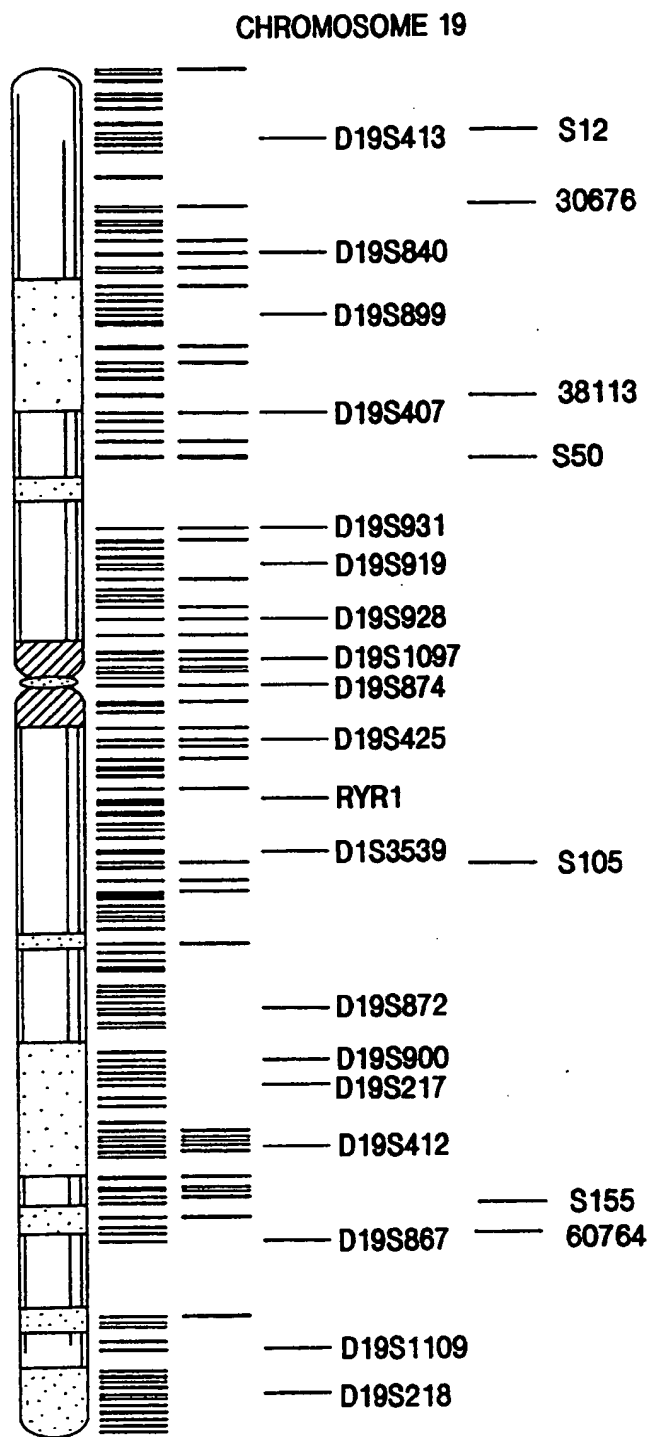
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SEQ ID NO:31 &lt;2099855H1 Incyte&gt;

GTGGCAACCAGCTCTTAGGCGTGGGAGAGGCCTACAGGCCCAAGGATATGGTGTCCGAAT  
CCAGGATGCTGGAGTTTATCTGCTGTATAGCCAGGTCCTGTTTCAAGACGTGACTTTCAC  
CATGGGTCAGGTGGTGTCTCGAGAAGGCCAAGGAAGGCAGGAGACTCTATTCCGATGTAT  
AAGAAGTATGCCCTCCCACCCGGACCGGGCCTACAACAGCTGCTATAGCGCAGGTGTCTT  
CCATTTACNCCAAGGGGATATTCTGAGTGTCTATAA

FIG. 31

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**FIG. 32**

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## CHROMOSOME 11

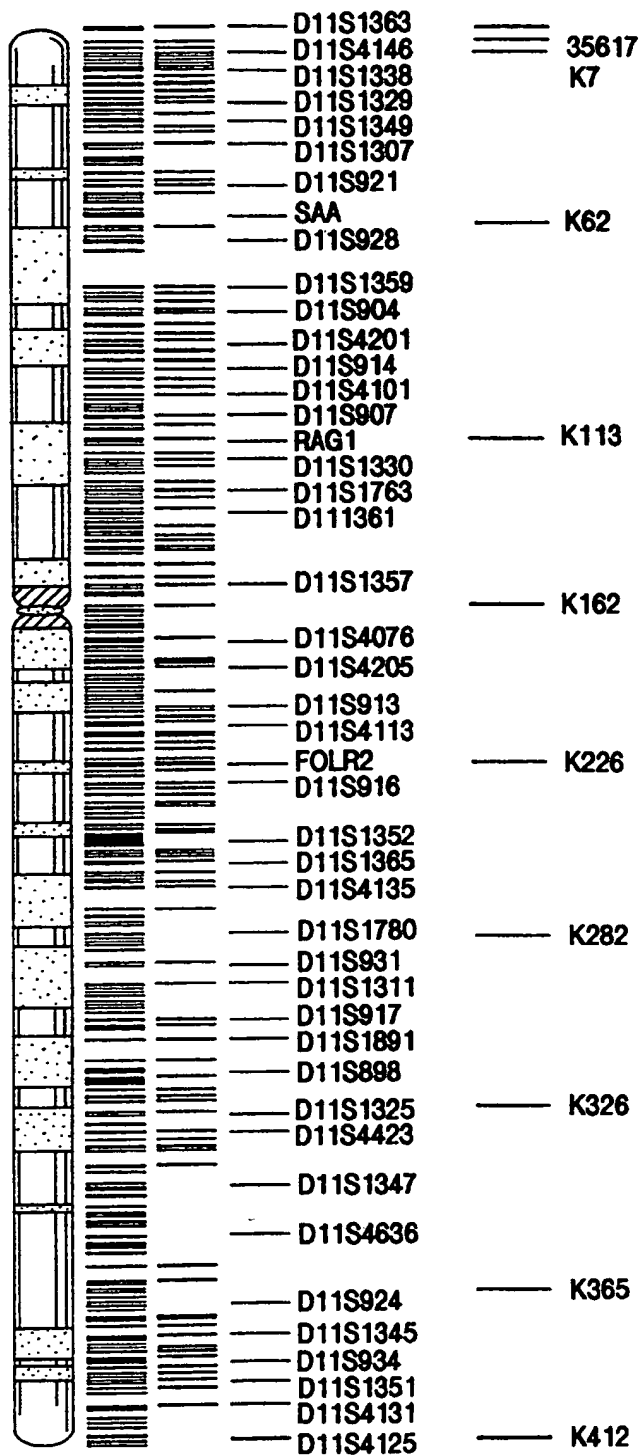
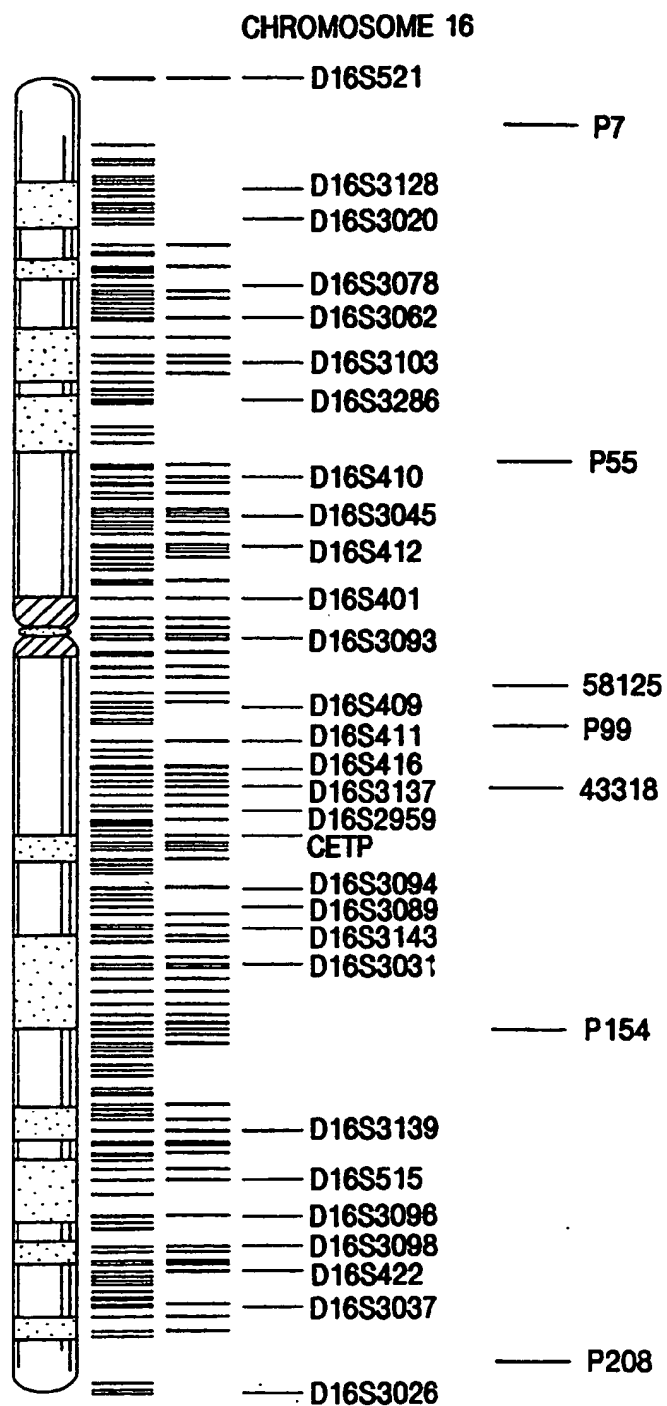


FIG. 33

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**FIG. 34**

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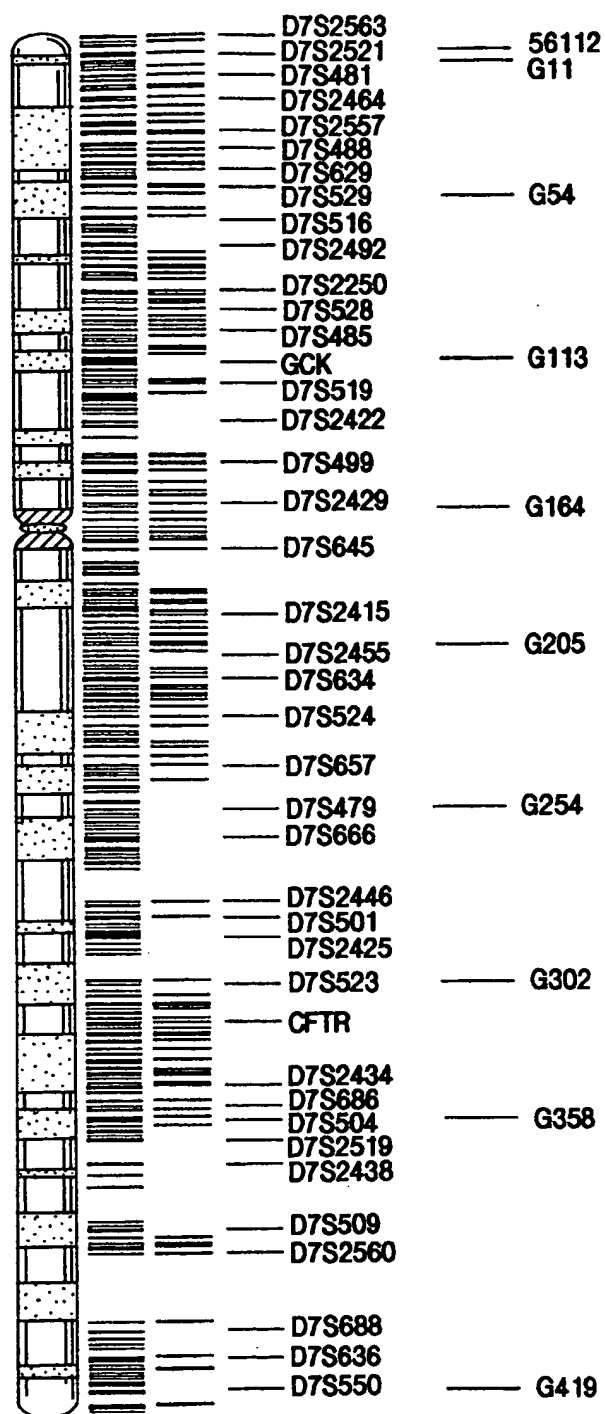
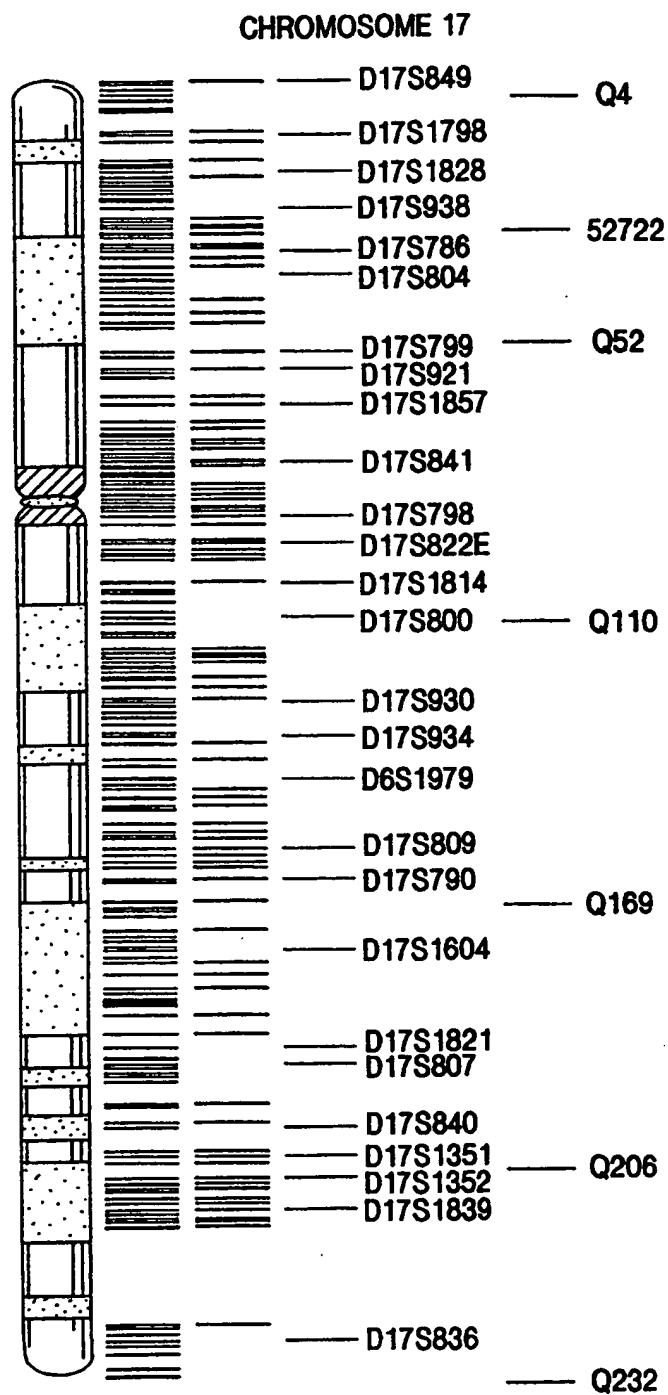
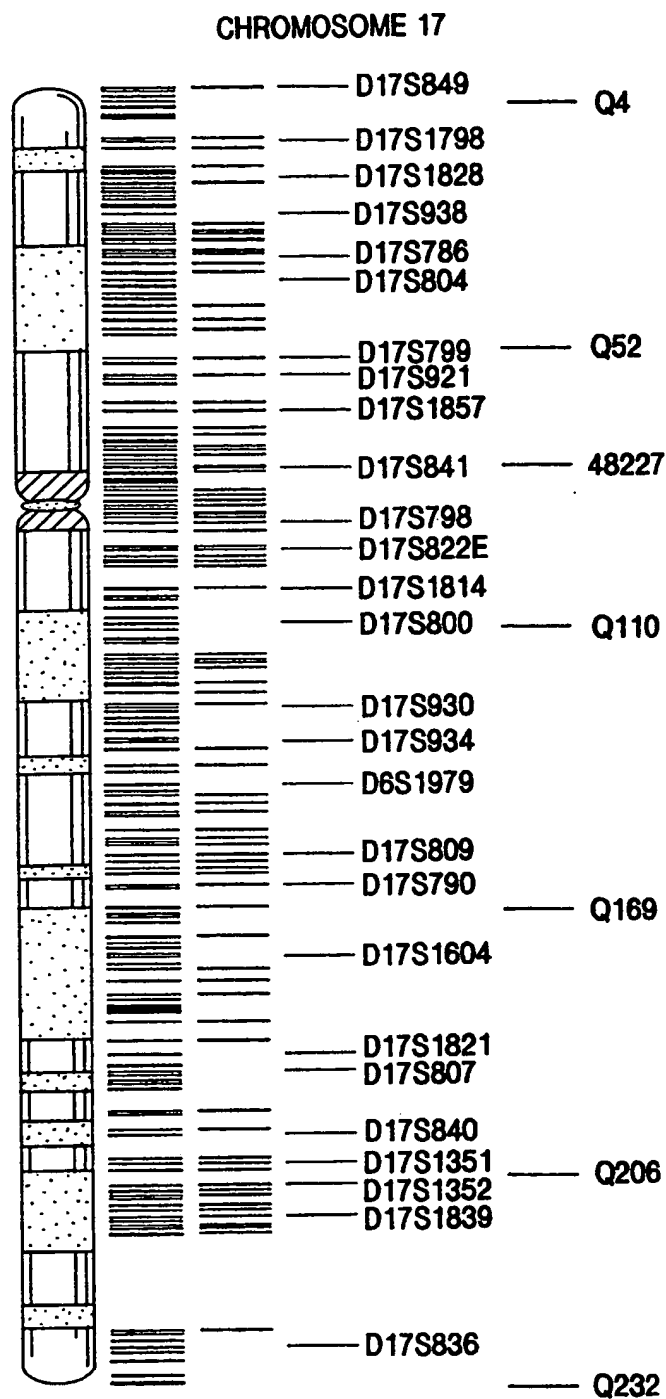


FIG. 35

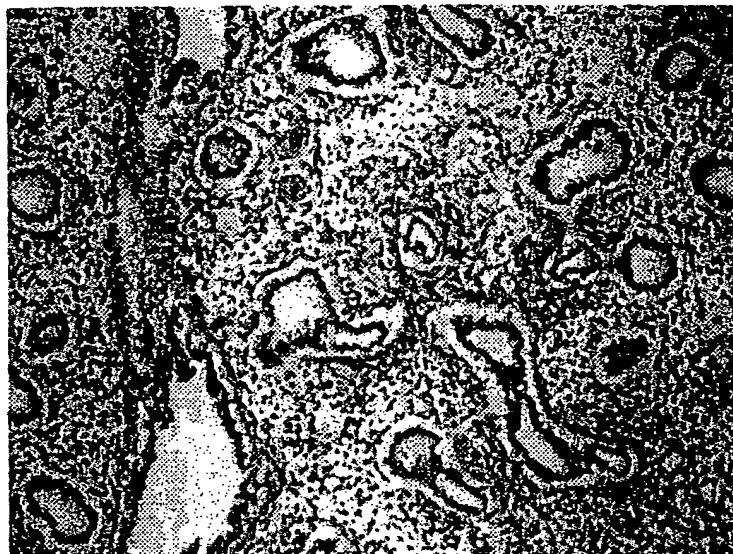
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**FIG. 36A**

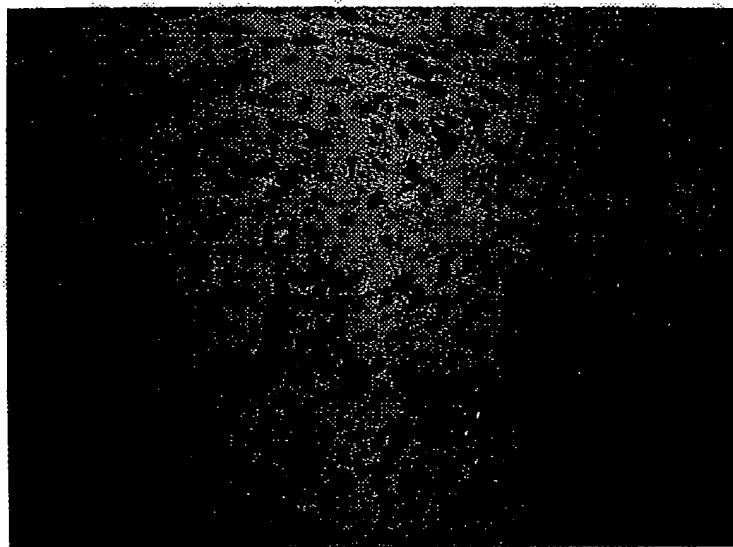
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**FIG. 36B**

**FIG. 37**



**FIG. 38**

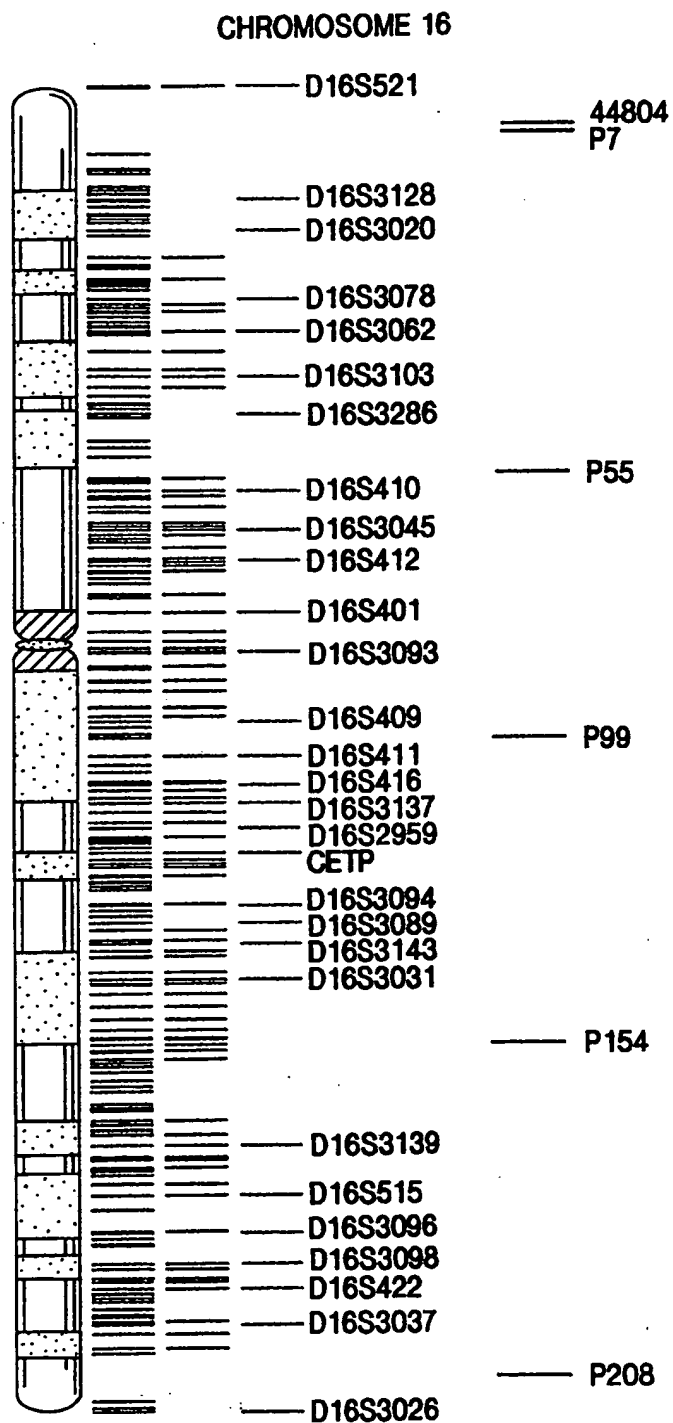


**FIG. 39**





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**FIG. 40**

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13ter.1(c) and Rule 39)

Applicant's or agent's file reference <b>P2509</b>	<b>IMPORTANT DECLARATION</b>	Date of mailing (day/month/year) <b>01/09/1999</b>
International application No. <b>PCT/US 99/ 00106</b>	International filing date (day/month/year) <b>05/01/1999</b>	(Earliest) Priority date (day/month/year) <b>05/01/1998</b>
International Patent Classification (IPC) or both national classification and IPC		<b>C07K18/30, A61K39/395, G01N33/577 G01N33/574</b>
Applicant <b>GENENTECH, INC. et al.</b>		


This International Searching Authority hereby declares, according to Article 17(2)(a), that no international search report will be established on the international application for the reasons indicated below

1. ☐ The subject matter of the international application relates to:
  - a. ☐ scientific theories.
  - b. ☐ mathematical theories
  - c. ☐ plant varieties.
  - d. ☐ animal varieties.
  - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
  - f. ☐ schemes, rules or methods of doing business.
  - g. ☐ schemes, rules or methods of performing purely mental acts.
  - h. ☐ schemes, rules or methods of playing games.
  - i. ☐ methods for treatment of the human body by surgery or therapy.
  - j. ☐ methods for treatment of the animal body by surgery or therapy.
  - k. ☐ diagnostic methods practised on the human or animal body.
  - l. ☐ mere presentations of information.
  - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:
 

☐ the description
 ☐ the claims
 ☐ the drawings
3. ☒ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out:
 

☒ the written form has not been furnished or does not comply with the standard.

☒ the computer readable form has not been furnished or does not comply with the standard.
4. Further comments: see FURTHER INFORMATION sheet PCT/ISA/203

Name and mailing address of the International Searching Authority  European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  <b>Barbara Klaver</b>
--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 203**

The claims of underlying application have, according to PCT Rule 13ter.1c, not been searched since the sequence listing as present in the description does not comply with WIPO standard ST25 prescribed in the administrative instructions under Rule 5.2, since the sequences have not been furnished neither on paper form nor in machine readable form as provided for in the same instructions and since the applicant has not remedied the disclosed deficiencies within the time limit fixed in the invitation to PCT Rule 13ter.1a.